Search Strategy

FILE 'USPATFULL' ENTERED AT 19:29:56 ON 24 JUN 2003

		E	PREVELIGE P E/IN
L1	1	S	E4
L2	25015	S	(HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L3	17172	S	L2 AND (GAG OR PR55GAG OR CA OR CAPSID OR MA OR MATRIX OR NC
L4	2510	S	L3 AND (VIRION ASSEMBLY OR MATURATION)
L5	89	S	L4 AND GAG/CLM
L6	65	S	L4 AND (CA/CLM OR CAPSID/CLM)
L7	56	S	L6 NOT L5
L8	75	S	L4 AND (MA/CLM OR MATRIX/CLM)
L9	68	S	L8 NOT (L5 OR L6)
L10	16	S	L4 AND (NC/CLM OR NUCLEOCAPSID/CLM)
L11	65	S	L4 AND (VIRION ASSEMBLY/CLM OR MATURATION/CLM OR CAPSIDS/CLM)
L12	55	S	L11 NOT (L6 OR L8 OR L10)
L13	3	S	L4 AND (SCREENING ASSAY/CLM)

FILE 'MEDLINE' ENTERED AT 19:54:26 ON 24 JUN 2003

		E	PREVELIGE P E/AU
L14	43	S	E3-E7
L15	3	S	L14 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L16	131819	S	(HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L17	8532	S	L16 AND (GAG OR PR55GAG OR CA OR CAPSID OR MA OR MATRIX OR NC
L18	876	S	L17 AND (ASSEMB? OR MATUR?)
L19	386	S	L18 AND (VIRION? OR CAPSIDS)
L20	58	S	L19 AND (ANTIVIRAL? OR SCREENING OR INHIBITION)
L21	60	S	L17 AND (VIRION ASSEMBLY OR VARION MATURATION OR CAPSID ASSEM
L22	1436	S	L17 AND (PURIFICATION)
L23	43	S	L22 AND SOLUBLE
L24	1630	S	L17 AND (IN VITRO)
L25	119	S	L24 AND ASSEMBLY
L26	100	S	L25 NOT (L21 OR L23)
L27	98	S	L26 NOT L14
L28	5	S	L17 AND (GUANIDINE)

FILE 'WPIDS' ENTERED AT 21:15:40 ON 24 JUN 2003

		E	PREVELIGE P E/IN
L29	1	S	E3
L30	15488	S	(HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L31	1414	S	L30 AND (GAG OR PR55GAG OR CA OR CAPSID OR MA OR MATRIX OR NC
L32	73	S	L31 AND (ASSEMBL? OR MATURAT?)
L33	16	S	L32 AND (IN VITRO)

L1 ANSWER 1 OF 1 USPATFULL
2001:194113 Method of monitoring HIV assembly and maturation.
Prevelige,, Peter E., JR., Birmingham, AL, United States
US 2001036627 A1 20011101
APPLICATION: US 2001-800240 A1 20010306 (9)
PRIORITY: US 2000-186981P 20000306 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods to characterize the structure, stability, and intersubunit interfaces between the matrix, capsid, and nucleocapsid domains of the Gag polyprotein during HIV capsid assembly and maturation. A method of screening for compounds that promote or inhibit viral assembly and maturation is disclosed. A novel mass spectrometry based approach to measure hydrogen/deuterium exchange profiles is also disclosed. Quantitative data resulted from these studies may lead to well defined capsid assembly assays that can be adapted for rapid antiviral drug screening.

CLM What is claimed is:

1. A method of screening for a compound that modulates viral assembly and maturation comprising the steps of: maintaining viral structural protein in a soluble form; triggering assembly of said viral structural protein; contacting said viral structural protein with a candidate compound or a control compound that does not inhibit viral assembly; and monitoring viral assembly, wherein an increase or decrease of viral assembly in the presence of said candidate compound compared to control compound indicates said candidate compound promotes or inhibits viral assembly respectively.

2. The method of claim 1, wherein said viral assembly and maturation is HIV-1 assembly and maturation.

3. The method of claim 2, wherein said viral structural protein is selected from the group consisting of matrix protein, capsid protein, nucleocapsid protein and gag protein of HIV-1.

4. The method of claim 1, wherein said viral structural protein is maintained in a soluble form through the use of pertubant.

5. The method of claim 4, wherein said perturbant is NaCl.

6. The method of claim 5, wherein said NaCl is in a concentration of from about 1 M to about 4 M.

7. The method of claim 4, wherein said perturbant is GuHCl.

8. The method of claim 7, wherein said GuHCl is in a concentration of from about 1 M to about 6 M.

9. The method of claim 1, wherein said assembly of viral protein is triggered by rapid removal of pertubant.

10. The method of claim 9, wherein said rapid removal of pertubant is by dilution.

11. The method of claim 3, wherein said candidate compound is selected from the group consisting of protein, peptide derived from the HIV-1 Gag polyprotein and a non-peptide small molecule.

12. The method of claim 1, wherein said monitoring of viral assembly is by a method selected from the group consisting of measuring turbidity measuring fluorescence and physical separation of the polymerized viral protein.

L5 ANSWER 1 OF 89 USPATFULL
2003:152911 HIV capsid assembly-associated compositions and method.
Lingappa, Vishwanath R., San Francisco, CA, UNITED STATES
Lingappa, Jaisri R., Seattle, WA, UNITED STATES
UC Regents (U.S. corporation)
US 2003104577 A1 20030605
APPLICATION: US 2002-40206 A1 20020102 (10)
PRIORITY: US 1997-39309P 19970207 (60)
DOCUMENT TYPE: Utility; APPLICATION.

AB A cell-free method for translation and assembly of retroviral, particularly HIV, capsid and capsid intermediates is disclosed. Also disclosed are novel HIV capsid assembly intermediates and novel host proteins which bind to such assembly intermediates. The invention also includes a screening method for compounds that alter retrovirus capsid assembly, and a method of treating HIV using compounds which inhibit the HIV capsid assembly pathway.

CLM What is claimed is:
1. A method of isolating an HIV capsid intermediate, said method comprising the steps of: combining HIV Gag Pr55 mRNA with a cell-free protein translation mixture containing myristoyl coenzyme A; incubating said translation mixture for a period of time sufficient to assemble Gag Pr55 mRNA translation products into immature HIV capsids; identifying said capsid intermediates on a linear sucrose gradient; and isolating said capsid intermediates by immunoprecipitation with antibodies specific for HIV Gag, whereby isolated capsid intermediates are obtained.

2. The method according to claim 1, wherein the amount of said myristoyl coenzyme A is about 0.1 to 100 micromolar.

3. The method according to claim 1, wherein said cell-free extract contains a detergent sensitive fraction from eukaryotic cells.

4. The method according to claim 1, wherein said cell-free extract contains a detergent-insensitive fraction from eukaryotic cells.

5. The method according to claim 1, wherein said cell-free extract contains an ATP sensitive fraction from eukaryotic cells.

6. A method of isolating an HIV capsid intermediate, said method comprising the steps of: combining HIV Gag Pr55 mRNA with a cell-free protein translation mixture containing myristol coenzyme A present in a concentration ranging from about 0. 1 to 100 micromolar, wherein said cell free mixture contains a detergent-insensiteve or a detergent sensitive fraction from eukaroytic cells; incubating said translation mixture for a period of time sufficient to assemble Gag Pr55 mRNA translation products into immature HIV capsids; identifying said capsid intermediates on a linear sucrose gradient; and isolating said capsid intermediates by immunoprecipitation with antibodies specific for HIV Gag, whereby isolated

capsid intermediates are obtained.

7. A method of identifying chaperone proteins involved in HIV capsid assembly, said method comprising the steps of: denaturing affinity purified capsid intermediate complexes so that said complexes are separated into chaperone proteins and capsid proteins; removing separated capsid proteins with monoclonal antibodies specific for capsid proteins leaving a mixture of chaperone proteins; isolating individual chaperone proteins from said mixture; sequencing said individual chaperone proteins, and comparing the sequences of said individual chaperone proteins to known sequences of host proteins, whereby the identity of host proteins that are involved in HIV capsid assembly are obtained.

8. A method of identifying chaperone proteins bound to HIV capsid intermediates produced in a mammalian cell, said method comprising the steps of: sequencing a human homologue to said chaperone protein identified in the cell-free system according to claim 7 using degenerate primers; expressing said human homologue in the cell-free system that has been immunodepleted for cell-free chaperone proteins wherein said sequence of human homologue is cloned into an expression vector; and measuring capsid formation in a cell-free translation system programmed with HIV Gag compared to a cell-free translation system programmed with HIV Gag that has not been immunodepleted, whereby comparable amounts of HIV capsid formation identifies chaperone protein produced in mammalian cells that bind to HIV capsid intermediates.

9. A method of identifying chaperone proteins bound to HIV capsid intermediates produced in a mammalian cell, said method comprising the steps of: sequencing a human homologue to said chaperone protein identified in the cell-free system according to claim 7 using degenerate primers; expressing said human homologue in HIV infected mammalian cells that are stably transfected with a dominant negative HP68 mutant wherein said sequence of human homologue is cloned into an expression vector; and measuring HIV viral release from said cells compared to cells not stabely stransfected with a dominant negative HP68 mutant, whereby restoraion of HIV viral release identifies chaperone proteins produced in mammalian cells that bind to HIV capsid intermediates.

10. A method of identifying conformers of host chaperone proteins that bind to HIV capsid intermediates, said method comprising the steps of: contacting isolated host proteins having an amino acid sequence substantially similar to a host chaperone protein with a plurality of monoclonal antibodies that bind to said host protein; identifying from said plurality of monoclonal antibodies those that bind to a subset of said host proteins and do not bind to said host chaperone proteins; isolating said host cell proteins so identified; and determining whether said conformer functions to facilitate assembly of HIV capsid intermediates, whereby conformers that so function are identified as conformers of said host chaperone protein.

11. A method of identifying a functional HP68 conformer, said method comprising the steps of: (a) isolating an RNase L inhibitor in cells not infected with HIV that does not bind to HIV Gag; (b) isolating HP68 that binds HIV Gag in cells producing HIV Gag; (c) expressing said RNase L inhibitor in a cell-free translation system that has been

immunodepleted for HP68, is programmed with HIV Gag mRNA and comprises an expression vector comprising a nucleic acid sequence encoding said RNase L inhibitor; (d) expressing said HP68 in a cell-free translation system that has been immunodepleted for HP68, is programmed with HIV Gag mRNA and comprises an expression vector comprising a nucleic acid sequence encoding said HP68; and (e) comparing capsid formation in step (c) to capsid formation in step (d), whereby a difference in amount of HIV capsids produced is indicative of a conformer of HP68 specific for HIV capsid formation.

12. A method of producing monoclonal antibodies to a conformer of a host protein that is involved in assembly of immature HIV capsids, said method comprising the steps of: immunizing knockout mice with a host chaperone protein, wherein said knockout mice have a non-functional gene that no longer codes for said conformer and lack the ability to produce said protein; producing hybridoma cells from spleens of said mice; screening said hybridoma cells for production of antibodies to both a native and a denatured conformer of said host chaperone protein; and propagating hybridoma cells producing antibodies that bind substantially specifically to said host chaperone protein and not to conformers of said host chaperone protein that do not bind Gag and do not facilitate HIV capsid assembly, whereby antibodies to native and denatured said protein or peptide conformer of interest are produced.

13. Monoclonal antibodies produced according to the method of claim 12.

14. Binding fragments to said conformer derived from monoclonal antibodies produced according to the method of claim 12.

15. A method of identifying a binding site between a host chaperone protein and an intermediate in HIV capsid assembly, said method comprising the steps of: obtaining a conformational epitope map of said host protein using monoclonal antibodies produced according to the method claim 12; obtaining a conformational epitope map of HIV Gag using monoclonal antibodies specific for Gag; obtaining a conformational epitope map of HIV capsid intermediates using monoclonal antibodies specific for HIV Gag and antibodies produced according to the method of claim 12; and comparing conformational epitope maps of HIV Gag, capsid intermediate complex and said conformer, whereby the binding site on said conformer for HIV Gag is identified and the binding site on HIV Gag for said conformer is isdentified.

16. A method for identifying compounds that interfere with HIV capsid assembly by specifically binding to and preventing said conformer from binding to HIV Gag, said method comprising the steps of: (a) screening databases for compounds that bind to the binding site identified according to the method of claim 15, whereby potential compounds are obtained; (c) screening said potential compounds for test compounds that bind substantially specifically to a host chaperone protein for HIV capsid assembly but not to conformers of said host chaperone proteins that do not bind HIV Gag; (d) screening said test compounds in a cell free translation system, wherein efficacy is measured by a decrease in HIV capsid production; and (f) further screening said compounds for ability to block HIV capsid formation in mammalian cells infected with HIV, whereby compounds that block HIV capsid formation are identified.

17. A method for identifying compounds that interfere with HIV capsid assembly by specifically binding to and preventing said conformer from binding to HIV Gag, said method comprising the steps of: a) expressing HIV Gag in a mammalian cell; b) identification of co-localization of HIV Gag and HP68 using immunofluorescence in said mammalian cells, and; c) screening said potential compounds for test compounds that interfere with co-localization of HP68 and Gag in said mammalian cells whereby compounds that interfer with HIV capsid assembly are identified by a diffuse staining pattern of HP68.

18. The method according to the method of claim 17, wherein said compounds do not cause toxcicity or upregulate host stress proteins in said mammalian cells.

19. A method of identifying a binding site between a host chaperone protein and an intermediate in HIV capsid assembly, said method comprising the steps of: obtaining a conformational epitope map of said host protein using monoclonal antibodies produced according to the method claim 12; obtaining a conformational epitope map of HIV Vif using monoclonal antibodies specific for Vif; obtaining a conformational epitope map of HIV capsid intermediates using monoclonal antibodies specific for HIV Vif and antibodies produced according to the method of claim 12; and comparing conformational epitope maps of HIV Vif, capsid intermediate complex and said conformer, whereby the binding site on said conformer for HIV Vif is identified and the binding site on HIV Vif for said conformer is isdentified.

20. A method for identifying compounds that interfere with HIV capsid assembly by specifically binding to and preventing said conformer from binding to HIV Vif, said method comprising the steps of: (a) screening databases for compounds that bind to the binding site identified according to the method of claim 15, whereby potential compounds are obtained; (c) screening said potential compounds for test compounds that bind substantially specifically to a host chaperone protein for HIV capsid assembly but not to conformers of said host chaperone proteins that do not bind HIV Vif, (d) screening said test compounds in a cell free translation system, wherein efficacy is measured by a decrease in HIV capsid production; and (g) further screening said compounds for ability to block HIV capsid formation in mammalian cells infected with HIV, whereby compounds that block HIV capsid formation are identified.

21. The method according to the method of claim 20, wherein said compounds do not cause toxcicity or upregulate host stress proteins in said mammalian cells.

22. A method for establishing a profile of host protein HIV capsid assembly chaperones and their conformers in a population of individuals infected with HIV, wherein said profile is relative to specific HIV characteristics, said method comprising the steps of: compiling a conformer profile of host protein HIV capsid assembly chaperones and their conformers in individual members of said population, wherein said individual members produce HIV virions; and establishing a relationship between said conformer profiles of said individual members and specific characteristics of HIV in said individual members, whereby a

population profile of conformers relative to specific HIV characteristics is obtained.

23. A method for selecting a treatment to administer to a individual infected with HIV, said method comprising the steps of: determining a conformer profile of host protein HIV capsid assembly chaperones and their conformers of said individual; comparing said conformer profile of said patient to a conformer population profile obtained according to the method of claim 22; and selecting as a method of treatment for said individual a method of treatment that was successful for treatment of individual members of said population having a substantially similar conformer profile, whereby a treatment based on a conformer profile is selected for said individual.

24. HIV capsid intermediates produced by a cell free system comprising: a) HIV Gag Pr55 mRNA; b) cell-free extract, amino acids, transfer RNA (tRNA), ribosomes and an energy source; c) a concentration of myristoyl coenzyme A about 0.1 to 100 micromolar; wherein said capsid intermediates are selected from the group consisting of proteins having a buoyant density of about 10S, about 80S, about 150S and about 500S.

25. The HIV capsid intermediates produced according to claim 24, wherein said intermediates comprise HIV capsid proteins and host chaperone proteins.

26. The HIV capsid intermediates produced according to claim 24, wherein said intermediates comprise Gag and HP68.

27. The HIV capsid intermediates produced according to claim 24, wherein said intermediates comprise Vif and HP68.

28. The HIV capsid intermediates produced according to claim 24, wherein said intermediates comprise HP68 that binds to Gag and Vif but does not bind to RNase L.

29. A cell-free system for translation and assembly of an HIV capsid, comprising a cell-free translation mixture, an mRNA molecule encoding a Gag Pr55 protein derived from human immunodeficiency virus (HIV), and myristoyl coenzyme A.

30. The cell-free translation system of claim 29, which further includes a detergent-sensitive fraction derived from eukaryotic cell membranes.

31. The cell-free translation system of claim 29, which further includes a eukaryotic cell component characterized by insensitivity to a concentration of at least 0.5% (wt/vol) "NIKKOL" detergent.

32. The cell-free translation system of claim 29, wherein said system further includes HIV genomic RNA or a fragment thereof.

33. The cell-free translation system of claim 29, which further includes (i) a DNA molecule which encodes HIV Gag Pr55, (ii) an RNA polymerase for synthesizing said mRNA, and (iii) sufficient concentrations of nucleotides ATP, UTP, GTP, and CTP to support such mRNA synthesis.

34. The cell-free translation system of claim 29, wherein said HIV Gag mRNA encodes a mutant defective in assembly.

35. A method of producing an HIV capsid intermediate in a cell-free system, comprising adding to a cell-free protein translation mixture which contains a cell-free extract, amino acids, transfer RNA (tRNA), ribosomes and an energy source: (i) an mRNA molecule encoding an HIV Pr55 Gag protein, and (ii) a concentration of myristoyl coenzyme A that is greater than about 0.1 micromolar, to form a reaction mixture; incubating said reaction mixture for a period of time sufficient to assemble Gag Pr55 mRNA translation products into an immature HIV capsid
.

36. The method of claim 35, wherein said reaction mixture is supplemented with (iii) a detergent-sensitive fraction derived from eukaryotic cell membranes, and (iv) a eukaryotic cell component characterized by insensitivity to a concentration of at least 0.5% (wt/vol) "NIKKOL" detergent.

37. The method of claim 35, wherein said reaction mixture is supplemented with host protein HP68 or a homolog thereof

38. The method of claim 35, which further includes adding to said reaction mixture an HIV genomic RNA molecule or a fragment thereof

39. The method of claim 35, which further includes adding to said reaction mixture a Gag Pr55 DNA transcript and a transcription mixture containing an RNA polymerase and ribonucleotides ATP, UTP, GTP and CTP effective to produce said Gag mRNA in said cell-free mixture.

40. An isolated HIV capsid intermediate selected from a group of HIV capsid intermediates having buoyant densities selected from the group of about 10S, about 80S, about 150S and about 500S, wherein said buoyant densities are measured by sedimentation in a linear sucrose density gradient ranging from 15% to 60% sucrose.

41. A method of selecting a compound capable of altering HIV capsid assembly, comprising adding a test compound to a reaction mixture which includes (i) a cell-free translation mixture that includes a cell-free extract, tRNA, ribosomes, amino acids and an energy source, (ii) an mRNA molecule encoding HIV Gag Pr55, (iii) myristoyl coenzyme A, present at a concentration greater than about 0.1 micromolar, measuring capsid assembly in the presence of said test compound, comparing assembly in the absence of said test compound to assembly in the presence of said compound, selecting the compound as a compound capable of altering HTV capsid assembly if assembly measured in the absence of said compound is significantly different than assembly measured in the presence of said compound.

42. The method of claim 41, wherein said measuring of capsid assembly includes measuring formation of assembly intermediates.

43. A host cell protein, comprising: a peptide region having the sequence presented as SEQ ID NO: 2, specific immunoreactivity with monoclonal antibody 23 c, and an apparent molecular weight of about 68 kilodaltons, wherein said protein associates with HIV capsid intermediates produced by the cell-free translation system of claim 29.

44. The host cell protein of claim 43, wherein said protein is characterized by at least 75% amino acid sequence identity to HP 68.

45. The host cell protein of claim 43, which is derived from wheat germ extract and which is identified as HP68.

46. A method of inhibiting HIV capsid formation in a cell, comprising adding to the cell a compound selected for its ability to inhibit HIV capsid formation in a cell-free translation system consisting essentially of (i) a cell-free translation mixture which contains a cell-free extract, tRNA, ribosomes, amino acids and an energy source, (ii) an mRNA molecule encoding a HIV capsid assembly protein Pr55, and (iii) myristoyl coenzyme A.

47. The method of claim 46, wherein said compound is selected for its ability to block association of the host protein of claim 41 or sequence homologs thereof with an HIV capsid intermediate.

48. A method of selecting a compound capable of altering HIV capsid assembly in cells, comprising adding a test compound to cells that are forming retroviral capsids, measuring the quantity and nature of capsid assembly intermediates formed within cells in the presence of said test compound, comparing the quantity and nature of assembly intermediates formed within cells in the absence of said test compound to said quantity and nature of intermediates formed in the presence of test compound, selecting the compound as a compound capable of altering formation of HIV assembly intermediates if the quantity or nature of intermediates measured in the presence of said compound is significantly different than the quantity or nature of intermediates measured in the absence of said compound.

49. The method of claim 48, wherein said selected retrovirus is HIV, and said measuring of said capsid formation is accomplished by measuring association of HP68 or a homolog thereof with an HIV capsid intermediate.

50. A method for encapsidating genomic HIV RNA or fragments thereof, comprising adding said RNA or RNA fragments to a cell-free translation system as defined in claim 29, and incubating said system for a period of time sufficient to complete said translation and assembly reaction.

L5 ANSWER 18 OF 89 USPATFULL
2002:254168 HIV immunoassays using gag polypeptides.
Luciw, Paul A., Davis, CA, United States
Dina, Dino, San Francisco, CA, United States
Steimer, Kathelyn, El Cerrito, CA, United States
Pescador, Ray Sanchez, Oakland, CA, United States
George-Nascimento, Carlos, Danville, CA, United States
Parkes, Deborah, Oakland, CA, United States
Hallewell, Rob, San Francisco, CA, United States
Barr, Philip J., Orinda, CA, United States
Truett, Martha, Oakland, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 6458527 B1 20021001
APPLICATION: US 1993-83391 19930628 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Polynucleotide sequences are provided for the diagnosis of the presence of retroviral infection in a human host associated with lymphadenopathy syndrome and/or acquired immune deficiency syndrome, for expression of polypeptides and use of the polypeptides to prepare antibodies, where both the polypeptides and antibodies may be employed as diagnostic reagents or in therapy, e.g., vaccines and passive immunization. The sequences provide detection of the viral infectious agents associated with the indicated syndromes and can be used for expression of antigenic polypeptides.

CLM What is claimed is:

1. In an immunoassay to detect the presence of antibodies to a human immunodeficiency virus (HIV) in a human sample comprising contacting said sample with an HIV gag antigen capable of binding anti-HIV antibodies in AIDS patient sera and determining whether antibodies are bound to said gag antigen, the improvement comprising employing as said gag antigen either a synthetic polypeptide or a recombinant polypeptide, wherein said gag antigen comprises an amino acid sequence of at least 7 contiguous amino acids selected from the gag ORF shown in FIG. 4, and wherein said recombinant polypeptide is the expression product of cellular hosts transformed by a heterologous expression vector comprising a DNA sequence encoding said recombinant polypeptide under the control of transcription and translation initiation and termination regulatory sequences functional in said cellular hosts.

2. A method of detecting antibodies to a human immunodeficiency virus (HIV) in a human sample comprising: (a) providing a solid support having bound thereto a polypeptide comprising an amino acid sequence of a least 7 contiguous amino acids selected from the gag ORF shown in FIG. 4, wherein said amino acid sequence is capable of binding to anti-HIV antibodies in AIDS patient sera, wherein said polypeptide is either a synthetic polypeptide or a recombinant polypeptide, wherein said recombinant polypeptide is the expression product of cellular hosts transformed by a heterologous expression vector comprising a DNA sequence encoding said recombinant polypeptide under the control of transcription and translation initiation and termination regulatory sequences functional in said hosts; (b) contacting said solid support with said human sample to provide a sample-contacted support; (c) washing said sample-contacted support to provide a washed support; and (d) determining whether human antibodies are bound to said washed support.

3. The method of claim 1 wherein said cellular hosts are microorganisms.

4. The method of claim 2 wherein said cellular hosts are microorganisms.

5. The method of claim 3 wherein said microorganisms are E. coli.

6. The method of claim 4 wherein said microorganisms are E. coli.

7. The method of claim 3 wherein said microorganisms are S. cerevisiae.

8. The method of claim 2 wherein said microorganisms are S. cerevisiae.

9. The method of claim 1 wherein said cellular hosts are mammalian cells.

10. The method of claim 2 wherein said cellular hosts are mammalian

cells.

11. The method of claim 1 wherein said gag antigen is not glycosylated.

12. The method of claim 2 wherein said polypeptide is not glycosylated.

13. The method of claim 1 wherein said human sample comprises serum.

14. The method of claim 2 wherein said human sample comprises serum.

15. The method of claim 2 wherein step (d) comprises contacting said washed support with labeled antibodies to human Ig and the specific binding of said labeled antibodies to said washed support is measured.

16. The method of claim 15 wherein said labeled antibodies bound to said washed support are measured by an enzyme label.

17. The method of claim 1 wherein said gag antigen is selected from the group consisting of p25gag, p16gag, and p53gag.

18. The method of claim 2 wherein said contiguous amino acid sequence is selected from the group consisting of p25gag, p16gag, and p53gag shown in FIG. 4.

19. An article of manufacture adapted for use in an immunoassay for antibodies to a human immunodeficiency virus (HIV) comprising a solid support having bound thereto a polypeptide comprising an amino acid sequence of at least 7 contiguous amino acids selected from the gag ORF shown in FIG. 4, wherein said amino acid sequence is capable of binding to anti-HIV antibodies in AIDS patient sera, wherein said polypeptide is either a synthetic polypeptide or a recombinant polypeptide, wherein said recombinant polypeptide is the expression product of cellular hosts transformed by a heterologous expression vector comprising a DNA sequence encoding said recombinant polypeptide under the control of transcription and translation initiation and termination regulatory sequences functional in said cellular hosts.

20. The article of manufacture of claim 19 wherein said gag antigen is not glycosylated.

21. The article of manufacture of claim 19 wherein said cellular hosts are microorganisms.

22. The article of manufacture of claim 21 wherein said microorganisms are E. coli.

23. The article of manufacture of claim 21 wherein said microorganisms are S. cerevisiae.

24. The article of manufacture of claim 19 wherein said cellular hosts are mammalian cells.

25. The method of claim 1 wherein said gag antigen is a synthetic polypeptide.

26. A polypeptide prepared by chemical synthesis comprising an amino acid sequence of at least 7 contiguous amino acids selected from the gag ORF shown in FIG. 4, wherein said amino acid sequence is capable of binding to anti-HIV antibodies in AIDS patient

sera.

27. The immunoassay of claim 1 wherein said contiguous amino acid sequence is at least 15 amino acid residues.

28. The method of claim 2 wherein said contiguous amino acid sequence is at least 15 amino acid residues.

29. The method of claim 12 wherein said contiguous amino acid sequence is at least 15 amino acid residues.

30. The method of claim 18 wherein said contiguous amino acid sequence is at least 15 amino acid residues.

31. The article of manufacture of claim 19 wherein said contiguous amino acid sequence is at least 15 amino acid residues.

32. The article of manufacture of claim 25 wherein said contiguous amino acid sequence is at least 15 amino acid residues.

33. The polypeptide of claim 26 wherein said contiguous amino acid sequence is at least 15 amino acid residues.

L5 ANSWER 22 OF 89 USPATFULL
2002:214438 Method of identifying modulators of HIV-1 Vpu and Gag interaction with U binding protein (Ubp).
Panganiban, Antonito T., Albuquerque, NM, UNITED STATES
Callahan, Michael A., Hamilton, MT, UNITED STATES
Handley, Mark A., Madison, WI, UNITED STATES
US 2002115830 A1 20020822
APPLICATION: US 2002-90378 A1 20020304 (10)
PRIORITY: US 1998-83567P 19980430 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A preparation of U binding protein (Ubp) and a gene sequence encoding Ubp and an anti-Ubp antibody are disclosed. An assay to identify modulators of Ubp/Vpu interaction and Gag/Ubp interaction is also disclosed.
CLM What is claimed is:
1. A preparation of U binding protein (Ubp).

2. A gene sequence encoding Ubp.

3. An assay to identify modulators of the Ubp/Vpu interaction, comprising the steps of (a) exposing Vpu and Ubp together in the presence of a candidate inhibitor under conditions in which Vpu and Ubp can interact when an inhibitor is not present, and (b) determining whether Vpu/Ubp interaction occurs.

4. The assay of claim 3 wherein the interaction is measured in vivo.

5. The assay of claim 3 wherein the interaction is measured in vitro.

6. The assay of claim 3 wherein the modulator is an inhibitor.

7. An assay for determining whether a candidate compound modulates the interaction between Gag and Ubp comprising the steps of (a) exposing Ubp and Gag in the presence of a candidate compound under conditions in which Ubp and Gag will interact when an inhibitor is not present, and (b) determining whether Gag and

Ubp interact.

8. The assay of claim 7 wherein the interaction is measured in vivo.

9. The assay of claim 7 wherein the interaction is measured in vitro.

10. The assay of claim 7 wherein the modulator is an inhibitor.

11. An inhibitor of Ubp/Vpu interaction.

12. The inhibitor of claim 11 wherein the inhibitor comprises a fragment of Ubp protein.

13. An inhibitor of the Gag/Ubp interaction.

14. The inhibitor of claim 13 wherein the inhibitor is a fragment of Ubp protein.

15. An anti-Ubp antibody.

16. A method of creating fragments of Ubp protein comprising examining SEQ ID NO:2 and synthesizing peptide fragments contained within SEQ ID NO:2.

17. A method of detecting members of the Ubp superfamily comprising examining SEQ ID NO:1 and constructing nucleic acid probes designed to specifically hybridize with Ubp homologs in non-human species.

18. A method of detecting members of the Ubp superfamily comprising examining SEQ ID NO:1 and constructing nucleic acid probes designed to specifically hybridized with Ubp homologs present in human cells.

L5 ANSWER 30 OF 89 USPATFULL
2002:99577 Chimeric viral proteins.
Srinivisan, Alagarsamy, Glen Mills, PA, UNITED STATES
Koprowski, Hilary, Wynnewood, PA, UNITED STATES
US 2002052478 A1 20020502
APPLICATION: US 2001-817413 A1 20010326 (9)
PRIORITY: US 1997-43380P 19970403 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Chimeric viral proteins and nucleic acid constructs that code for them and are useful as therapeutic agents are disclosed.

CLM What is claimed is:
1. A nucleic acid construct comprising a nucleotide sequence coding for a chimeric viral protein comprising: (a) a protein of a virus; and (b) a polypeptide of said virus, said polypeptide joined by a peptide linkage to said viral protein in said chimeric protein, said polypeptide not normally joined by said peptide linkage to said protein in said virus or in cells infected by said virus.

2. The nucleic acid construct of claim 1 that is a DNA construct.

3. The nucleic acid construct of claim 1 comprising a nucleotide sequence coding for: (a) a protein of a virus, said protein not comprising a site for cleavage by a proteolytic enzyme of said virus; (b) a polypeptide proteolytic cleavage site of said virus, said cleavage site being a site for cleavage by a proteolytic enzyme of said virus;

such that said protein is covalently linked by a peptide linkage to said polypeptide proteolytic cleavage site.

4. The nucleic acid construct of claim 3, wherein the virus is an animal virus or a human virus.

5. The nucleic acid construct of claim 4, wherein the virus is a human virus.

6. The nucleic acid construct of claim 5 wherein the virus is selected from the group consisting of: herpes simplex virus type I, herpes simplex virus type II, human cytomegalovirus, human herpes virus type, and human immunodeficiency virus (HIV).

7. The nucleic acid construct of claim 6 wherein the virus is human immunodeficiency virus (HIV).

8. The nucleic acid construct of claim 7, wherein the protein of the virus is not a capsid protein of the virus.

9. The nucleic acid construct of claim 8, wherein the viral protein is the vpr protein.

10. The nucleic acid construct of claim 8, wherein the polypeptide proteolytic cleavage site corresponds to an amino acid sequence found in the Gag or Gag-Pol precursor proteins of HIV .

11. The nucleic acid construct of claim 1 comprising a nucleotide sequence coding for: (a) a protein of a virus, said first protein not being a protein that forms a dimeric proteolytic enzyme of said virus; (b) a dimer interface polypeptide sequence of an enzyme of said virus, said sequence being one by which monomers of said enzyme combine to form the active dimeric enzyme, such that said first protein is covalently linked by a peptide linkage to said dimer interface polypeptide sequence.

12. The nucleic acid construct of claim 11, wherein the enzyme is selected from the group consisting of: protease, DNA polymerase and ribonucleotide reductase.

13. The nucleic acid construct of claim 12, wherein the dimer interface polypeptide sequence is that of the HIV protease.

14. A nucleic acid construct comprising a nucleotide sequence coding for a chimeric viral protein comprising: (a) a non-capsid protein of a virus, the non-capsid protein not comprising a polypeptide proteolytic cleavage site for cleavage by a proteolytic enzyme; (b) a polypeptide of the virus, the polypeptide joined by a peptide linkage to the viral protein in said chimeric protein, wherein the polypeptide is not normally joined by the peptide linkage to the non-capsid protein in said virus or in cells infected by the virus.

15. The nucleic acid construct of claim 13 wherein the polypeptide has the cleavage site for cleavage by the proteolytic enzyme.

16. The nucleic acid construct of claim 14 wherein the cleavage site being a site for cleavage by the proteolytic enzyme of the virus.

17. The nucleic acid construct of claim 13 wherein the virus is an animal virus or a human virus.

18. The nucleic acid construct of claim 16 wherein the virus is a human virus.

19. The nucleic acid construct of claim 17 wherein the virus is selected from the group consisting of: herpes simplex virus type I, herpes simplex virus type II, human cytomegalovirus, human herpes virus type, and human immunodeficiency virus (HIV).

20. The nucleic acid construct of claim 18 wherein the virus is human immunodeficiency virus (HIV).

21. The nucleic acid construct of claim 19 wherein the polypeptide proteolytic cleavage site corresponds to an amino acid sequence found in the Gag or Gag-Pol precursor proteins of HIV .

22. A chimeric viral protein of claim 20, wherein the non-capsid protein is the vpr protein.

23. A process of interfering with the growth of a virus in an animal or human, said process comprising administering to the animal or human a nucleic acid construct of claim 1.

24. The process of claim 23 wherein the animal or human has not been infected with the virus.

25. The process of claim 23 wherein the animal or human has been infected with the virus.

26. The process of claim 23 wherein the construct is administered so as to become integrated into a cell of said animal or human.

27. The process of claim 26 wherein the cell is selected from the group consisting of a bone marrow cell and a blood cell.

28. The process of claim 27 wherein the cell is a lymphocyte.

29. The process of interfering with the growth of a virus in an animal or human, said process comprising administering to the animal or human a nucleic acid construct of claim 14.

L5 ANSWER 32 OF 89 USPATFULL
2002:37504 METHOD OF IDENTIFYING MODULATORS OF HIV-1 VPU AND GAG INTERACTION WITH U BINDING PROTEIN (UBP).
PANGANIBAN, ANTONITO T., ALBUQUERQUE, NM, UNITED STATES
CALLAHAN, MICHAEL A., HAMILTON, MT, UNITED STATES
HANDLEY, MARK A., CAMBRIDGE, WI, UNITED STATES
US 2002022221 A1 20020221
APPLICATION: US 1999-301978 A1 19990429 (9)
PRIORITY: US 1998-83567P 19980430 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A preparation of U binding protein (Ubp) and a gene sequence encoding

Ubp and an anti-Ubp antibody are disclosed. An assay to identify modulators of Ubp/Vpu interaction and Gag/Ubp interaction is also disclosed.

CLM What is claimed is:
1. A preparation of U binding protein (Ubp).

2. A gene sequence encoding Ubp.

3. An assay to identify modulators of the Ubp/Vpu interaction, comprising the steps of (a) exposing Vpu and Ubp together in the presence of a candidate inhibitor under conditions in which Vpu and Ubp can interact when an inhibitor is not present, and (b) determining whether Vpu/Ubp interaction occurs.

4. The assay of claim 3 wherein the interaction is measured in vivo.

5. The assay of claim 3 wherein the interaction is measured in vitro.

6. The assay of claim 3 wherein the modulator is an inhibitor.

7. An assay for determining whether a candidate compound modulates the interaction between Gag and Ubp comprising the steps of (a) exposing Ubp and Gag in the presence of a candidate compound under conditions in which Ubp and Gag will interact when an inhibitor is not present, and (b) determining whether Gag and Ubp interact.

8. The assay of claim 7 wherein the interaction is measured in vivo.

9. The assay of claim 7 wherein the interaction is measured in vitro.

10. The assay of claim 7 wherein the modulator is an inhibitor.

11. An inhibitor of Ubp/Vpu interaction.

12. The inhibitor of claim 11 wherein the inhibitor comprises a fragment of Ubp protein.

13. An inhibitor of the Gag/Ubp interaction.

14. The inhibitor of claim 13 wherein the inhibitor is a fragment of Ubp protein.

15. An anti-Ubp antibody.

16. A method of creating fragments of Ubp protein comprising examining SEQ ID NO:2 and synthesizing peptide fragments contained within SEQ ID NO:2.

17. A method of detecting members of the Ubp superfamily comprising examining SEQ ID NO:1 and constructing nucleic acid probes designed to specifically hybridize with Ubp homologs in non-human species.

18. A method of detecting members of the Ubp superfamily comprising examining SEQ ID NO:1 and constructing nucleic acid probes designed to specifically hybridized with Ubp homologs present in human cells.

L5 ANSWER 43 OF 89 USPATFULL
2000:101880 Chimeric Gag pseudovirions.

Tobin, Gregory J., Frederick, MD, United States
Gonda, Matthew A., Newtown Square, PA, United States
The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 6099847 20000808
APPLICATION: US 1997-857385 19970515 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides, inter alia, recombinant chimeric nucleic acids encoding a Gag-fs-fusion partner fusion protein; a pseudovirion comprising a retroviral Gag protein and a fusion partner, wherein the fusion partner is present in a Gag -fs-fusion partner fusion protein; an immunogenic composition comprising a pseudovirion; a Gag-fs-fusion partner fusion protein; and a method of making the pseudovirions of the present invention.

CLM What is claimed is:
1. A recombinant chimeric nucleic acid, comprising: a retroviral gag sequence; a target nucleic acid sequence derived from a nucleic acid encoding a fusion partner selected from the group consisting of Env, an interleukin, TNF, GM/CSF, a nonretroviral viral antigen and a cancer antigen; wherein the gag and target sequences are transcribed from a single start site of transcription, and wherein the gag and target sequences are in different reading frames; and, a frame-shift site.

2. The recombinant chimeric nucleic acid of claim 1, wherein the target nucleic acid sequence is derived from a nucleic acid encoding a fusion partner selected from the group consisting of IL-1, IL-2, IL-4, IL-6, MART-1, gp 100, tyrosinase, bcl-1, bcl-2, c-myc, int-2, hst-1, ras, p53, prostate-specific membrane antigen, papilloma virus protein L1, protein kinase C., and G proteins.

3. The recombinant chimeric nucleic acid of claim 1, wherein the frame shift site is derived from a site selected from the group consisting of a retroviral frame shift site, a retrotransposon frame shift site, a human astrovirus frame shift site, a mouse intracisternal particle frame shift site, an HERV frame shift site, a Ty element frame shift site, and an optimized synthetic frameshift site.

4. A recombinant chimeric gag-env nucleic acid, comprising: a retroviral gag sequence; a retroviral env sequence; wherein the gag and env sequences are transcribed from a single start site of transcription, and wherein the gag and env sequences are in different reading frames; and, a retroviral frame-shift site derived from a retroviral gag-pol frame shift site.

5. The recombinant nucleic acid of claim 4, wherein the env sequence encodes approximately the carboxyl 65% of Env protein.

6. The recombinant nucleic acid of claim 4, wherein the nucleic acid further comprises a pol sequence.

7. The recombinant nucleic acid of claim 4, wherein the nucleic acid is a subsequence in a baculoviral vector.

8. The recombinant nucleic acid of claim 4, wherein the nucleic acid is competent to produce pseudovirions in an insect cell.

9. The recombinant nucleic acid of claim 4, wherein the nucleic acid is

competent to produce pseudovirions in an insect cell, and wherein the nucleic acid hybridizes under stringent conditions to HIV Gag-fs-SU.

10. The recombinant nucleic acid of claim 4, wherein the nucleic acid is HIV Gag-fs-SU or a conservative variation thereof.

11. The recombinant nucleic acid of claim 4, wherein the nucleic acid is HIV Gag-fs-SU.

12. The recombinant nucleic acid of claim 4, wherein the nucleic acid is a subsequence in a baculoviral vector, wherein the vector is competent to transduce an insect cell.

13. The recombinant nucleic acid of claim 4, wherein the gag and env sequences are derived from HIV.

14. The recombinant nucleic acid of claim 4, wherein the nucleic acid further comprises a polyhedrin promoter.

15. The recombinant nucleic acid of claim 4, wherein the nucleic acid further comprises an SV 40 polyadenylation site.

16. A pseudovirion comprising a retroviral Gag protein and a fusion partner, wherein the fusion partner is present in a Gag -fs-fusion partner fusion protein.

17. The pseudovirion of claim 16, wherein the fusion partner is derived from a protein selected from the group consisting of an interleukin, TNF, GM/CSF, a nonretroviral viral antigen, a cancer antigen and a molecule involved in signal transduction.

18. The pseudovirion of claim 17, wherein the fusion partner is derived from a protein selected from the group consisting of IL-1, IL-2, IL-4, IL-6, MART-1, gp 100, tyrosinase, bcl-1, bcl-2, c-myc, int-2, hst-1, ras, p53, prostate-specific membrane antigen, papilloma virus protein L1, protein kinase C., and G proteins.

19. The pseudovirion of claim 16, wherein the fusion partner is derived from a retroviral Env protein.

20. The pseudovirion of claim 16, wherein the pseudovirion is noninfectious.

21. The pseudovirion of claim 19, wherein the Env protein domain is present primarily in the interior of the pseudovirion.

22. The pseudovirion of claim 19, wherein the Gag-fs-Env fusion protein is the Gag-fs-SU fusion protein, or a conservative modification thereof.

23. The pseudovirion of claim 19, wherein the Gag-fs-Env fusion protein is the Gag-fs-SU fusion protein.

24. The pseudovirion of claim 19, wherein the Env fusion partner is present in a Gag-fs-Env fusion protein, and wherein Gag protein is separately present in the fusion protein and as an independent protein.

25. The pseudovirion of claim 19, wherein the pseudovirion is made by transducing an insect cell with a baculovirus vector, which vector

encodes a Gag-fs-Env protein.

26. The pseudovirion of claim 19, wherein the pseudovirion, when administered as an immunogenic composition in mice, elicits a CTL response against Env, but does not elicit antibodies which recognize Env.

27. An immunogenic composition comprising a pseudovirion comprising a retroviral Gag protein and a retroviral fusion partner, wherein the fusion partner is present in a Gag-fs-fusion partner fusion protein and wherein the fusion partner is derived from a retroviral Env protein.

28. The immunogenic composition of claim 27, wherein the immunogenic composition, when administered to mice, elicits a CTL response against Env, but does not elicit antibodies against Env.

29. A particulate vaccine comprising a pseudovirion comprising a retroviral Gag protein and a retroviral fusion partner, wherein the fusion partner is present in a Gag-fs-fusion partner fusion protein and wherein the fusion partner is derived from a retroviral Env protein.

30. The particulate vaccine of claim 29, wherein the vaccine, when administered to mice, elicits a CTL response against Env, but does not elicit antibodies against Env.

31. A fusion protein comprising a retroviral Gag sequence, a translation reading frame switching sequence and a fusion partner.

32. The fusion protein of claim 31, wherein the fusion partner is a retroviral Env amino acid subsequence.

33. The fusion protein of claim 31, wherein the fusogenic partner is selected from the group consisting of Env, an interleukin, TNF, GM/CSF, a nonretroviral viral antigen, a cancer antigen and a molecule involved in signal transduction.

34. The fusion protein of claim 31, wherein the fusogenic partner is selected from the group consisting of IL-1, IL-2, IL-4, IL-6, MART-1, gp 100, tyrosinase, bcl-1, bcl-2, c-myc, int-2, hst-1, ras, p53, prostate-specific membrane antigen, papilloma virus protein L1, protein kinase C. and G proteins.

35. The fusion protein of claim 32, wherein the Env amino acid subsequence comprises the carboxyl 65% of a retroviral Env protein.

36. The fusion protein of claim 32, wherein the Env amino acid subsequence is derived from HIV.

37. The fusion protein of claim 31, wherein the translation reading frame switching sequence comprises sequences derived from the N-terminus of a retroviral Pol protein.

38. A method of making a pseudovirion comprising expressing a nucleic acid encoding a Gag-fs-fusion partner fusion protein in a cell, wherein the cell translates the nucleic acid into a first protein comprising a Gag sequence, and a second protein comprising a gag sequence and a fusogenic partner.

39. The method of claim 38, wherein the fusogenic partner comprises an

env sequence.

40. The method of claim 38, wherein the cell is an insect cell.

41. The method of claim 38, wherein the method further comprises the step of purifying the pseudovirion.

42. A pseudovirion comprising a retroviral Gag protein and a fusion partner, wherein the fusion partner is present in Gag -fs-fusion partner fusion protein and wherein the fusion partner is derived from retroviral Env protein.

43. A fusion protein comprising a retroviral Gag sequence, a translation reading frame switching sequence and a retroviral Env amino acid subsequence.

L5 ANSWER 46 OF 89 USPATFULL
2000:47067 Self-assembled, defective, nonself-propagating viral particles.
Mazzara, Gail P., Winchester, MA, United States
Panicali, Dennis L., Acton, MA, United States
Roberts, Bryan, Cambridge, MA, United States
Gritz, Linda R., Somerville, MA, United States
Stallard, Virginia, Sequim, WA, United States
Mahr, Anna, Natick, MA, United States
Therion Biologics, Corp., Cambridge, MA, United States (U.S. corporation)
US 6051410 20000418
APPLICATION: US 1997-958906 19971028 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Recombinant viral vectors which coexpress heterologous polypeptides capable of assembling into defective nonself-propagating viral particles are disclosed. The viral vectors as well as the viral particles can be used as immunogens and for targeted delivery of heterologous gene products and drugs.

CLM What is claimed is:
1. A pox virus having inserted therein, at least two DNA sequences from a single species of lentivirus DNA sequences wherein one of the lentivirus DNA sequences is selected from the group consisting of the gag gene, gag-pol and portions thereof, such that the lentivirus DNA sequences express gag, gag-pol proteins, or portions thereof, referred to as said first lentivirus DNA sequence and a second lentivirus DNA sequence encoding another lentiviral protein, wherein the lentivirus proteins or portions thereof, self-assembled into defective, non-self-propagating lentivirus particles.

2. The pox virus of claim 1, wherein the pox virus is a vaccinia virus or a fowl pox virus.

3. A pox virus, said pox virus having inserted therein, at least two DNA sequences from a single species of human immunodeficiency virus (HIV) wherein one of the HIV DNA sequences is selected from the group consisting of the gag gene and gag-pol gene and said other HIV DNA sequence encodes another HIV protein, wherein the protein expressed by the gag gene or gag-pol gene and said other HIV DNA sequence self-assemble into defective, non-self propagating HIV particles.

4. The pox virus of claim 3, wherein the pox virus is a vaccinia virus or a fowl pox virus.

5. The pox virus of claim 4, wherein the pox virus is a vaccinia virus.

6. The pox virus of claim 3, wherein said other HIV DNA sequence is the env gene.

7. A vaccinia virus vector, said vaccinia virus vector is a first and a second chimeric gene inserted within the HindIII M region of the vaccinia virus vector, wherein the first chimeric gene comprises an HIV gag-pol gene, or a portion thereof, operatively linked to the 40K vaccinia promoter, and the second chimeric gene comprises a different HIV gene such that the gag, pol proteins, or portions thereof, are co-expressed in the eukaryotic host cell infected with the pox virus vector, and the gag, pol and other HIV proteins, or portions thereof, self-assemble into defective, non-self-propagating HIV particles.

L5 ANSWER 51 OF 89 USPATFULL
1999:75316 Non-infectious HIV particles and uses therefor.
Aldovini, Anna, Winchester, MA, United States
Young, Richard A., Winchester, MA, United States
Feinberg, Mark B., San Francisco, CA, United States
Trono, Didier, Solana Beach, CA, United States
Baltimore, David, New York, NY, United States
Whitehead Institute for Biomedical Research, Cambridge, MA, United States (U.S. corporation)
US 5919458 19990706
APPLICATION: US 1995-477081 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention related to constructs comprising mutant HIV genomes having an alteration in a nucleotide sequence which is critical for genomic RNA packaging and non-infectious, immunogenic HIV particles produced by expression of these constructs in mammalian cells. Cell lines which stably produce non-infectious, immunogenic HIV particles are also included. Prophylactic and therapeutic vaccines, diagnostic reagents, and related methods are further described.

CLM What is claimed is:
1. An immunogen comprising non-infectious HIV particles or antigenic portions thereof, in a physiologically acceptable vehicle, wherein the non-infectious HIV particles or antigenic portions thereof have a protein composition similar to that of a wild type HIV and lack HIV genomic RNA and are produced by a method comprising the steps of: a) transfecting a mammalian cell line with a construct which encodes non-infectious HIV particles and, when expressed in mammalian cells, produces only non-infectious HIV particles which have a protein composition similar to that of wild type HIV and lack HIV genomic RNA, the construct having an alteration of the wild type HIV genome, the alteration selected from the group consisting of: 1) deletion in the .psi. site, wherein the deletion is selected from the group consisting of: i) deletion of nucleotides 293 to 331, inclusive, and ii) deletion of nucleotides 293 to 313, inclusive; 2) an alteration in the gag gene which results in an alteration of the amino acid sequence of the encoded protein, the amino acid alteration selected from

the group consisting of: i) substitution of tyrosine for the first two cysteines of the 5' CysHis box; ii) substitution of tyrosine for the first two cysteines of the 3' CysHis box; iii) substitution of tyrosine for the first two cysteines of both CysHis boxes; iv) deletion of both CysHis boxes and the amino acid sequence between them; and v) alteration of the length of the amino acid sequence between the two CysHis boxes; and 3) deletion in the .psi. site and an alteration in the gag gene which results in an alteration of the amino acid sequence of the encoded protein, wherein: i) the deletion in the .psi. site is selected from the group consisting of: (a) deletion of nucleotides 293 to 331, inclusive; and (b) deletion of nucleotides 293 to 313, inclusive; and ii) the alteration in the gag gene is selected from the group consisting of: (a) substitution of tyrosine for the first two cysteines of the 5' CysHis box, (b) substitution of tyrosine for the first two cysteines of the 3' CysHis box; (c) substitution of tyrosine for the first two cysteines of both CysHis boxes; (d) deletion of both CysHis boxes and the amino acid sequence between them; and (e) alteration of the length of the amino acid sequence between the two CysHis boxes; and b) expressing the construct in the cell line, thereby producing non-infectious HIV particles which have a protein composition similar to that of wild type HIV and lack HIV genomic RNA.

2. An immunogen comprising non-infectious HIV-1 virions or antigenic portions thereof, in a physiologically acceptable vehicle, wherein the non-infectious HIV virions or antigenic portions thereof have a protein composition similar to that of wild type HIV and lack HIV genomic RNA and are produced by culturing a mammalian cell line which stably produces non-infectious HIV particles which have a protein composition similar to that of wildtype HIV and lack HIV genomic RNA, wherein the mammalian cell line comprises a mutant HIV genome stably integrated in the genome of said cell line and the mutant HIV genome comprises an alteration of the wild type HIV genome, the alteration selected from the group consisting of: 1) deletion in the .psi. site, wherein the deletion is selected from the group consisting of: i) deletion of nucleotides 293 to 331, inclusive; and ii) deletion of nucleotides 293 to 313, inclusive; 2) an alteration in the gag gene which results in an alteration of the amino acid sequence of the encoded protein, the amino acid alteration selected from the group consisting of: i) substitution of tyrosine for the first two cysteines of the 5' CysHis box; ii) substitution of tyrosine for the first two cysteines of the 3' CysHis box; iii) substitution of tyrosine for the first two cysteines of both CysHis boxes; iv) deletion of both CysHis boxes and the amino acid sequence between them; and v) alteration of the length of the amino acid sequence between the two CysHis boxes; and 3) deletion in the .psi. site and an alteration in the gag gene which results in an alteration of the amino acid sequence of the encoded protein, wherein: i) the deletion in the .psi. site is selected from the group consisting of: (a) deletion of nucleotides 293 to 331, inclusive; and (b) deletion of nucleotides 293 to 313, inclusive; and ii) the alteration in the gag gene is selected from the group consisting of: (a) substitution of tyrosine for the first two cysteines of the 5' CysHis box; (b) substitution of tyrosine for the first two cysteines of the 3' CysHis box; (c) substitution of tyrosine for the first two cysteines of both CysHis boxes; (d) deletion of both CysHis boxes and the amino acid sequence between them; and (e) alteration of the length of the amino acid sequence between the two CysHis boxes, thereby producing non-infectious HIV virions which have a protein composition similar to that of wild type HIV and lack HIV genomic RNA.

3. The immunogen of claim 1, wherein the construct in step a) is selected from the group consisting of pA3HXB and pA4HXB.

4. The immunogen of claim 1, wherein the construct in step a) is selected from the group consisting of: a) pA15HXB; b) pA14HXB; c) pA14-15HXB; and d) p.DELTA.CH1-2HXB.

5. The immunogen of claim 1, wherein the construct in step a) is selected from the group consisting of p.DELTA.PAC1 and p.DELTA.PAC-Hygro.

6. The immunogen of claim 1, wherein the construct in step a) further comprises an alteration selected from the group consisting of: a) an alteration which results in substitution of the envelope precursor cleavage site with VVQGEEFAVG (SEQ ID NO:9); b) deletion of the primer binding site; and c) a combination of 1) an alteration which results in substitution of the envelope precursor cleavage site with VVQGEEFAVG and 2) deletion of the primer binding site.

7. The immunogen of claim 6, wherein the construct is selected from the group consisting of p.DELTA.PAC2 and p.DELTA.PAC3.

8. The immunogen of claim 1, wherein the construct in step a) further comprises an SV40 origin of replication.

9. The immunogen of claim 1, wherein the alteration of the wildtype HIV genome in the construct in step a) further comprises a selectable marker gene in place of the nef gene.

10. The immunogen of claim 9, wherein said selectable marker gene encodes a selectable marker which is selected from the group consisting of neomycin resistance, hygromycin resistance and dihydrofolate reductase.

11. The immunogen of claim 1, wherein the construct in step a) further comprises an HIV env gene from another HIV strain or isolate which is substituted for a native HIV env gene of the construct.

L5 ANSWER 68 OF 89 USPATFULL
1998:75376 Screening method for the identification of compounds capable of abrogation HIV-1 gag-cyclophilin complex formation.
Luban, Jeremy, New York, NY, United States
Goff, Stephen P., Tenafly, NJ, United States
The Trustees of Columbia University in the City of New York, New York, NY, United States (U.S. corporation)
US 5773225 19980630
APPLICATION: US 1994-248357 19940524 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The human immunodeficiency virus type 1 (HIV-1) gag gene product is capable of directing the assembly of virion particles independent of other viral elements. The Gag protein also plays an important role during the early stages of viral replication. Employing the yeast two-hybrid system, a cDNA expression library was screened and two host proteins identified. These proteins, designated cyclophilins A and B (CyPsA and B), interacted specifically with the HIV-1 Gag polyprotein

Pr55.sup.gag. Glutathione S-transferase-CyP fusion proteins bind tightly to Pr55.sup.gag in vitro. Cyclosporin A (CsA) efficiently disrupts the Gag-CyPA binding interaction. The identification of novel compounds capable of abrogating this protein-protein interaction employing the disclosed screening assay will facilitate the development of HIV-1 antiviral agents.

CLM What is claimed is:

1. A method for identifying compounds capable of interfering with the formation of a complex between an HIV-1 Gag protein and a cyclophilin (CyP), which comprises the following steps: a) producing a CyP affinity fusion protein; b) pre-incubating a compound with the CyP affinity fusion protein of step (a); c) adding an HIV-1 Gag protein to the incubate of step (b) under conditions which permit Gag and the CyP affinity fusion protein to form a complex; d) contacting the incubate of step (c) with an affinity medium under conditions that enable the Gag-CyP affinity fusion protein complex to bind to said affinity medium; e) determining the amount of said Gag-cyclophilin affinity fusion protein complex formation by comparison to a control sample lacking said compound; wherein reduced binding of HIV-1 Gag to the cyclophilin affinity fusion protein is indicative of the ability of said compound to inhibit said complex formation.

2. The method of claim 1, wherein the CyP employed in the CyP affinity fusion protein is selected from the group consisting of CyP A, B, C, D, and combinations thereof.

3. The method of claim 1, wherein the CyP affinity fusion protein is a glutathione S-transferase-CyP (GST-CyP) fusion protein.

4. The method of claim 1, wherein the HIV-1 Gag protein is Pr55.sup.gag.

5. The method of claim 1, wherein the HIV-1 Gag protein is p24.

6. The method of claim 1, wherein the affinity medium comprises glutathione-agarose beads.

7. The method of claim 1, wherein the amount of said HIV-1 Gag-CyP affinity fusion protein complex formed is determined using monoclonal antibodies.

8. The method of claim 1, wherein the amount of said HIV-1 Gag-CyP affinity fusion protein complex formed is determined using polyclonal antibodies.

9. The method of claim 1, wherein the HIV-1 Gag protein is labeled with a detectable moiety selected from the group consisting of a fluorescent label, a radioactive label, and a chemiluminescent label.

10. The method of claim 1, wherein the HIV-1 Gag-CyP affinity fusion protein complex is purified and removed from the affinity medium and the amount of Gag protein ascertained.

11. A method for identifying compounds capable of interfering with the formation of a complex between a cyclophilin (CyP) and an HIV -1 Gag affinity fusion protein, which comprises the following steps: a) producing an HIV-1 Gag affinity fusion

protein; b) pre-incubating a compound with the HIV-1 Gag affinity fusion protein of step (a); c) adding a CyP to the incubate of step (b) under conditions which permit the CyP and the HIV-1 Gag affinity fusion protein to form a complex; d) contacting the incubate of step (c) with an affinity medium under conditions that enable the CyP-Gag affinity fusion protein complex to bind to said affinity medium; e) determining the amount of said CyP-Gag affinity fusion protein complex formation by comparison to a control sample lacking said compound; wherein reduced binding is indicative of the ability of said compound to inhibit CyP-HIV-1 Gag affinity fusion protein complex formation.

12. The method of claim 11, wherein the cyclophilin employed is selected from the group consisting of cyclophilin A, B, C, D, and combinations thereof.

13. The method of claim 11, wherein the HIV-1 Gag protein employed in the HIV-1 Gag affinity fusion protein is Pr55.sup.gag.

14. The method of claim 11, wherein the HIV-1 Gag protein employed in the HIV-1 Gag affinity fusion protein is p24.

15. The method of claim 11, wherein the affinity medium comprises glutathione-agarose beads.

16. The method of claim 11, wherein the amount of said CyP-Gag affinity fusion protein complex formed is determined using monoclonal antibodies.

17. The method of claim 11, wherein the amount of said CyP-Gag affinity fusion protein complex formed is determined using polyclonal antibodies.

18. The method of claim 11, wherein the CyP is labeled with a detectable moiety selected from the group consisting of a fluorescent label, a radioactive label, and a chemiluminescent label.

19. The method of claim 11, wherein the CyP-HIV-1 Gag affinity fusion protein complex is purified and removed from the affinity medium and the amount of CyP protein ascertained.

20. The method of claim 1, wherein the CyP employed in the CyP affinity fusion protein is CyP A.

21. The method of claim 11, wherein the CyP employed is CyP A.

L5 ANSWER 79 OF 89 USPATFULL
97:42784 Self assembled, defective, non-self-propagating lentivirus particles.
Mazzara, Gail P., Winchester, MA, United States
Panicali, Dennis L., Acton, MA, United States
Roberts, Bryan, Cambridge, MA, United States
Gritz, Linda R., Somerville, MA, United States
Stallard, Virginia, Sequim, WA, United States
Mahr, Anna, Natick, MA, United States
Therion Biologics, Incorporated, Cambridge, MA, United States (U.S. corporation)
US 5631154 19970520
APPLICATION: US 1993-18344 19930216 (8)

DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Recombinant avipox viral vectors which express heterologous polypeptides capable of assembling into defective nonself-propagating viral particles are disclosed. The recombinant avipox viruses can be used to produce significant amounts of the heterologous polypeptides in avian or non-avian cells. Preferably, the recombinant avipox virus is a fowlpox virus. The viral particles can also be used as immunogens and for targeted delivery of heterologous gene products and drugs.

CLM What is claimed is:

1. An avipox virus vector having inserted therein at least two DNA sequences from a single species of lentivirus, wherein one of the lentivirus DNA sequences is the env gene and the other of the lentivirus DNA sequences is selected from the group consisting of the gag gene and gag-pol gene, such that the lentivirus DNA sequences express either env and gag proteins, or express env and gag-pol proteins in a eukaryotic host cell co-infected with the two pox virus vectors, and the lentivirus proteins self-assemble into defective, non-self-propagating lentivirus particles.

2. Two avipox virus vectors, each avipox virus vector having inserted therein only one of either of two DNA sequences from a single species of lentivirus, wherein one of the lentivirus DNA sequences is the env gene and the other of the lentivirus DNA sequences is selected from the group consisting of the gag gene and gag-pol gene, such that the lentivirus DNA sequences express either env and gag proteins, or express env and gag-pol proteins in a eukaryotic host cell co-infected with the two pox virus vectors, and the lentivirus proteins self-assemble into defective, non-self-propagating lentivirus particles.

3. The avipox vector of claim 1, wherein the avipox is a fowlpox virus.

4. The avipox vectors of claim 2, wherein each avipox is a fowlpox virus.

5. The avipox vector of claim 3, wherein the lentivirus is Simian Immunodeficiency Virus (SIV) or Human Immunodeficiency Virus (HIV).

6. The avipox vectors of claim 4, wherein the lentivirus particle is Simian Immunodeficiency Virus (SIV) or Human Immunodeficiency Virus (HIV).

7. A self-assembled, defective non-self-propagating lentivirus particle produced by eukaryotic host cell infected with the avipox virus vector of claim 1.

8. A self-assembled, defective non-self-propagating lentivirus particle produced by eukaryotic host cell infected with the avipox virus vector of claim 2.

9. The avipox vector of claim 5 wherein the lentivirus is HIV.

10. The avipox vector of claim 6 wherein the lentivirus is HIV
.

11. A self-assembled, defective non-self-propagating lentivirus particle produced by eukaryotic host cell infected with the avipox virus vector

of claim 9.

12. A self-assembled, defective non-self-propagating lentivirus particle produced by eukaryotic host cell infected with the avipox virus vector of claim 10.

L9 ANSWER 65 OF 68 USPATFULL
97:15959 Method for identifying agents which block infection of cells by HIV.
Trono, Didier P., Solana Beach, CA, United States
Aiken, Christopher R., Encinitas, CA, United States
Swingler, Simon M., San Diego, CA, United States
Gallay, Philippe A., Solana Beach, CA, United States
The Salk Institute for Biological Studies, La Jolla, CA, United States (U.S. corporation)
US 5605802 19970225
APPLICATION: US 1994-316038 19940930 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB In accordance with the present invention, there are provided novel assays which allow the identification of compounds which block the ability of lentiviruses to infect non-dividing cells. Compounds discovered employing the invention methods can be employed to block the ability of HIV to infect non-dividing cells. In accordance with another aspect of the present invention, novel antibodies have been developed which are specifically immunoreactive with the phosphorylated form of HIV-1 MA. In accordance with yet another aspect of the present invention, novel kinases which phosphorylate HIV-1 MA have been discovered.

CLM What is claimed is:
1. A method for identifying compounds that inhibit tyrosine phosphorylation of human immunodeficiency virus type 1 Matrix protein (MA), said method comprising: exposing MA to tyrosine phosphorylation conditions independently in the presence, and in the absence, of test compound, detecting the level of tyrosine phosphorylation of MA upon exposure of MA to said test compound, and identifying those test compounds which cause a reduced level of tyrosine phosphorylation of MA as compounds that inhibit tyrosine phosphorylation of MA.

2. A method according to claim 1 wherein said MA is recombinantly produced.

3. A method according to claim 1 wherein said phosphorylation conditions comprise contacting said MA with a cell extract derived from human T cell lines, fibroblasts, epithelial cells or serum.

4. A method according to claim 1 wherein said test compound is selected from antibiotics, anti-fungal agents, anti-neoplastic agents, enzymatically active agents, immunostimulating agents or immunosuppressive agents.

5. A method according to claim 1 wherein MA is contacted with test compound at a concentration in the range of about 0.01 nM up to 10 .mu.M.

6. A method according to claim 1 wherein the phosphorylation of MA is detected employing ELISA, Western blot or uptake of

labelled phosphate.

7. A method for identifying compounds that inhibit tyrosine phosphorylation of human immunodeficiency virus type 1 Matrix protein (MA), said method comprising: exposing MA to tyrosine phosphorylation conditions in the presence of varying amounts of test compound, detecting the level of tyrosine phosphorylation of MA upon exposure of MA to varying amounts of test compound, and identifying those test compounds which cause a reduced level of tyrosine phosphorylation of MA as compounds that inhibit tyrosine phosphorylation of MA.

8. A method according to claim 7 wherein said MA is recombinantly produced.

9. A method according to claim 7 wherein said phosphorylation conditions comprise contacting said MA with a cell extract derived from human T cell lines, fibroblasts, epithelial cells or serum.

10. A method according to claim 7 wherein said test compound is selected from antibiotics, anti-fungal agents, anti-neoplastic agents, enzymatically active agents, immunostimulating agents or immunosuppressive agents.

11. A method according to claim 7 wherein MA is contacted with test compound at a concentration in the range of about 0.01 nM up to 10 .mu.M.

12. A method according to claim 7 wherein the phosphorylation of MA is detected employing ELISA, Western blot or uptake of labelled phosphate.

L10 ANSWER 10 OF 16 USPATFULL
2001:202380 Oligonucleotides which specifically bind retroviral nucleocapsid proteins.
Rein, Alan, Columbia, MD, United States
Casas-Finet, Jose, Gaithersburg, MD, United States
Fisher, Robert, Sharpsburg, MD, United States
Fivash, Matthew, Frederick, MD, United States
Henderson, Louis E., Mount Airy, MD, United States
The United States of America as represented by the Secretary of the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 6316190 B1 20011113
WO 9744064 19971127
APPLICATION: US 1999-180903 19990712 (9)
WO 1997-US8936 19970519 19990712 PCT 371 date 19990712 PCT 102(e) date
PRIORITY: US 1996-17128P 19960520 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides oligonucleotides which bind to retroviral nucleocapsid proteins with high affinity, molecular decoys for retroviral nucleocapsid proteins which inhibit viral replication, targeted molecules comprising high affinity oligonucleotides, assays for selecting test compounds, and related kits.

CLM What is claimed is:
1. A targeted molecule comprising an oligonucleotide which binds to a retroviral nucleocapsid protein with high affinity, and a

fusion partner, wherein the targeted molecule binds to the retroviral nucleocapsid protein with high affinity.

2. The targeted molecule of claim 1, wherein the fusion partner chemically reacts with the retroviral nucleocapsid protein, thereby reducing the ability of the nucleocapsid protein to package retroviral RNA.

3. The targeted molecule of claim 1, wherein the fusion partner is cytotoxic.

4. The targeted molecule of claim 1, wherein the fusion partner is a protein.

5. The targeted molecule of claim 1, wherein the oligonucleotide is a GT rich DNA oligonucleotide, or a GU rich RNA oligonuclotide.

6. The targeted molecule of claim 1, wherein the oligonucleotide is selected from the group consisting of a tetranucleotide, a pentanucleotide, a hexanucleotide, a heptanucleotide and an octanucleotide.

7. The targeted molecule of claim 1, wherein the oligonucleotide comprises a sequence selected from the group of sequences consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

8. The targeting molecule of claim 1, wherein the targeted molecule binds to HIV-1 nucleocapsid (NC) with high affinity.

9. The targeting molecule of claim 1, wherein the fusion partner is a label.

10. The targeting molecule of claim 1, wherein the targeting molecule further comprises a label.

11. The targeted molecule of claim 1, wherein the fusion partner is selected from the group consisting of: disulfides having the formula R--S--S--R; maleimides having the formula ##STR3## .alpha.-halogenated ketones having the formula ##STR4## nitric oxide and derivatives containing the NO group; hydrazides having the formula R--NH--NH--R; nitroso compounds having the formula R--NO; cupric ions and complexes containing Cu.sup.+2 ; ferric ions and complexes containing Fe.sup.+3 ; and alkylating agents; wherein R can be any atom or molecule, and X is a halogen selected from the group consisting of I, F, Br and Cl.

12. A recombinant nucleic acid which encodes an oligonucleotide which binds to a retroviral nucleocapsid protein with high affinity, wherein the nucleic acid comprises a promoter which directs expression of the oligonucleotide in a mammalian cell.

13. The nucleic acid of claim 12, wherein the nucleic acid is packaged in a viral vector.

14. The nucleic acid of claim 12, wherein the nucleic acid is packaged in a retroviral vector.

15. A cell comprising the nucleic acid of claim 12.

16. The cell of claim 15, wherein the cell is a human cell.

17. The cell of claim 15, wherein the cell is a human stem cell.

18. The cell of claim 15, wherein the cell is a human CD4+ cell.

19. A composition comprising a molecular decoy, the molecular decoy comprising an oligonucleotide which binds to a retroviral nucleocapsid protein with high affinity.

20. The composition of claim 19, wherein the molecular decoy is an oligonucleotide with a sequence selected from the group of sequences consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

21. The composition of claim 19, further comprising a protein selected from the group consisting of a nucleocapsid protein, and a Gag protein, wherein the protein is bound to the oligonucleotide.

22. The composition of claim 19, further comprising a pharmaceutical excipient.

23. An assay for detecting target molecules which inhibit binding of an oligonucleotide to a retroviral nucleocapsid protein comprising: providing a retroviral nucleocapsid protein; providing an oligonucleotide which binds to the retroviral nucleocapsid protein with high affinity; providing a target molecule; mixing the retroviral nucleocapsid protein, oligonucleotide and target molecule; and, measuring the inhibitory effect of the target molecule on oligonucleotide binding to the retroviral nucleocapsid protein.

24. The assay of claim 23, wherein the oligonuclcotide is labeled.

25. The assay of claim 23, wherein the target molecule is selected from the group consisting of oligonucleotides and peptides.

26. The assay of claim 23, wherein the oligonucleotide, target molecule and retroviral protein are mixed in an aqueous solution.

27. The assay of claim 23, wherein the inhibitory effect of the target molecule is measured by plasmon resonance.

28. The assay of claim 23, wherein the assay further comprises parallel analysis of a second target molecule by performing the steps of providing a retroviral nucleocapsid protein; providing an oligonucleotide which binds to the retroviral nucleocapsid protein with high affinity; providing a second target molecule; independently mixing the second target molecule with the retroviral nucleocapsid protein and oligonucleotide; and, measuring the inhibitory effect of the target molecule on oligonucleotide binding to the retroviral nucleocapsid protein.

29. The assay of claim 28, wherein the second target molecule, retroviral nucleocapsid protein and oligonucleotide are mixed in a well on a microtiter tray.

30. A method of detecting a nucleocapsid (NC) protein comprising binding an NC-specific oligonucleotide to the NC protein, thereby forming an NC -oligonuclcotide complex, and detecting the complex, thereby detecting

the NC protein.

31. The method of claim 30, wherein the oligonucleotide comprises a detectable label and detection of the NC-oligonucleotide complex is performed by detecting the detectable label.

32. The method of claim 30, wherein the NC protein is a component of an intact retrovirus.

33. The method of claim 30, wherein the NC protein is a Gag precursor protein.

34. The method of claim 30, wherein the NC protein is derived from HIV-1.

35. A method of purifying an NC protein comprising binding an NC-specific oligonucleotide to the NC protein, thereby forming an NC-oligonucleotide complex, and purifying the complex, thereby purifying the NC protein.

36. The method of claim 35, wherein the NC protein is a component of an intact retrovirus.

37. The method of claim 35, wherein the oligonucleotide is linked to a magnetic bead.

L15 ANSWER 1 OF 3 MEDLINE
2003001739 Document Number: 22396235. PubMed ID: 12507478. Identification of novel interactions in HIV-1 capsid protein assembly by high-resolution mass spectrometry. Lanman Jason; Lam TuKiet T; Barnes Stephen; Sakalian Michael; Emmett Mark R; Marshall Alan G; Prevelige Peter E Jr. (Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294-2170, USA.) JOURNAL OF MOLECULAR BIOLOGY, (2003 Jan 24) 325 (4) 759-72. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB The pleomorphic nature of the immature and mature HIV-1 virions has made it difficult to characterize intersubunit interactions using traditional approaches. While the structures of isolated domains are known, the challenge is to identify intersubunit interactions and thereby pack these domains into supramolecular structures. Using high-resolution mass spectrometry, we have measured the amide hydrogen exchange protection factors for the soluble capsid protein (CA) and CA assembled in vitro. Comparison of the protection factors as well as chemical crosslinking experiments has led to a map of the subunit/subunit interfaces in the assembled tubes. This analysis provides direct biochemical evidence for the homotypic N domain and C domain interactions proposed from cryo-electron microscopy image reconstruction of CA tubes. Most significantly, we have identified a previously unrecognized intersubunit N domain-C domain interaction. The detection of this interaction reconciles previously discrepant biophysical and genetic data.

L15 ANSWER 2 OF 3 MEDLINE
2002727135 Document Number: 22377458. PubMed ID: 12489858. Mapping of protein:protein contact surfaces by hydrogen/deuterium exchange, followed by on-line high-performance liquid chromatography-electrospray ionization Fourier-transform ion-cyclotron-resonance mass analysis. Lam TuKiet T; Lanman Jason K; Emmett Mark R; Hendrickson Christopher L; Marshall Alan G; Prevelige Peter E. (Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306, USA.) JOURNAL OF CHROMATOGRAPHY. A, (2002 Dec 20) 982 (1) 85-95. Journal code: 9318488. Pub. country: Netherlands. Language: English.

AB For protein complexes too large, uncrystallizable/insoluble, or low concentration for conventional X-ray diffraction or nuclear magnetic resonance analysis, the contact surface(s) may be mapped by comparing H/2H exchange rate (and thus solvent accessibility) of backbone amide hydrogens in free vs. complexed protein(s). The protein is first exposed to 2H2O, allowed to exchange for each of several reaction periods, and then digested with pepsin. The extent and rate of H/2H exchange is determined by measuring the increase in mass with H/2H exchange period for each of the peptides. Here, we present an experimental protocol that combines rapid (to minimize back-exchange) HPLC front-end separation with ultrahigh-resolution mass analysis (needed to distinguish the isotopic distributions of dozens of peptides simultaneously). The method is used to study the assembled human immunodeficiency virus type capsid protein (CA) and its soluble form.

L15 ANSWER 3 OF 3 MEDLINE
2002329867 Document Number: 22068019. PubMed ID: 12072491. Kinetic analysis of the role of intersubunit interactions in human immunodeficiency virus type 1 capsid protein assembly in vitro. Lanman Jason; Sexton Jennifer; Sakalian Michael; Prevelige Peter E Jr. (Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294-2170, USA.) JOURNAL OF VIROLOGY, (2002 Jul) 76 (14) 6900-8. Journal code: 0113724. ISSN: 0022-538X. Pub.

country: United States. Language: English.

AB The human immunodeficiency virus type 1 (HIV-1) capsid protein (CA) plays a crucial role in both assembly and maturation of the virion. Numerous recent studies have focused on either the soluble form of CA or the polymer end product of in vitro CA assembly. The CA polymer, in particular, has been used to study CA-CA interactions because it is a good model for the CA interactions within the virion core. However, analysis of the process of in vitro CA assembly can yield valuable insights into CA-CA interactions and the mechanism of core assembly. We describe here a method for the analysis of CA assembly kinetics wherein the progress of assembly is monitored by using turbidity. At pH 7.0 the addition of either of the isolated CA domains (i.e., the N or the C domain) to an assembly reaction caused a decrease in the assembly rate by competing for binding to the full-length CA protein. At pH 8.0 the addition of the isolated C domain had a similar inhibitory affect on CA assembly. However, at pH 8.0 the isolated N domain had no affect on the rate of CA assembly but, when mixed with the C domain, it alleviated the C-domain inhibition. These data provide biochemical evidence for a pH-sensitive homotypic N-domain interaction, as well as for an N- and C-domain interaction.

L20 ANSWER 1 OF 58 MEDLINE
2003174649 Document Number: 22549841. PubMed ID: 12662926.
Antiviral inhibition of the HIV-1 capsid protein. Tang Chun; Loeliger Erin; Kinde Isaac; Kyere Samson; Mayo Keith; Barklis Eric; Sun Yongnian; Huang Mingjun; Summers Michael F. (Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250-5398, USA.) JOURNAL OF MOLECULAR BIOLOGY, (2003 Apr 11) 327 (5) 1013-20. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB During the assembly stage of the human immunodeficiency virus (HIV) replication cycle, several thousand copies of the viral Gag polyprotein associate at the cell membrane and bud to form an immature, non-infectious virion. Gag is subsequently cleaved by the protease, which liberates the capsid proteins for assembly into the polyprotein shell of the central core particle (or capsid) of the mature virus. Viral infectivity is critically dependent on capsid formation and stability, making the capsid protein a potentially attractive antiviral target. We have identified compounds that bind to an apical site on the N-terminal domain of the HIV-1 capsid protein and inhibit capsid assembly in vitro. One compound, N-(3-chloro-4-methylphenyl)-N'-[2-[([5-[(dimethylamino)-methyl]-2-furyl]-methyl)-sulfanyl]ethyl]urea) (CAP-1), is well tolerated in cell cultures, enabling in vivo antiviral and mechanistic studies. CAP-1 inhibits HIV-1 infectivity in a dose-dependent manner, but does not interfere with viral entry, reverse transcription, integration, proteolytic processing, or virus production, indicating a novel antiviral mechanism. Significantly, virus particles generated in the presence of CAP-1 exhibit heterogeneous sizes and abnormal core morphologies, consistent with inhibited CA-CA interactions during virus assembly and maturation. These findings lay the groundwork for the development of assembly inhibitors as a new class of therapeutic agents for the treatment of AIDS.

L20 ANSWER 19 OF 58 MEDLINE
2000234623 Document Number: 20234623. PubMed ID: 10774590. Effect of

protease inhibitors on HIV-1 maturation and infectivity. Jardine D K; Tyssen D P; Birch C J. (Victorian Infectious Diseases Reference Laboratory, North Melbourne, Australia.) ANTIVIRAL RESEARCH, (2000 Jan) 45 (1) 59-68. Journal code: 8109699. ISSN: 0166-3542. Pub. country: Netherlands. Language: English.

AB The effects of HIV-1 protease inhibitors on proteolytic processing and infectivity of virions produced from lymphocytes chronically infected with the virus were studied. Protease inhibition was detected by the accumulation of the polyprotein precursors Pr55gag and Pr160gag-pol and their cleavage intermediates. Immunoblot analysis demonstrated that while the processing of Pr55gag was largely irreversible, cleavage of Pr160gag-pol proceeded once the inhibitor was removed, although it was not completed during 96 h of subsequent observation. Virions produced during exposure of cells to protease inhibitors regained some degree of infectivity post-withdrawal of the inhibitor, suggesting that the processing of Pr160gag-pol following drug withdrawal resulted in the production of those enzymes necessary to enable at least limited viral replication. When cells were exposed to a protease inhibitor for 72 h then the inhibitor withdrawn, a lag phase of up to 24 h occurred before these cells produced virions with equivalent infectivity to virus produced from cells not exposed to drug. These observations may reflect a clinical situation likely to occur as trough plasma concentrations of protease inhibitors fall below the IC100 for HIV , highlighting the need for adherence to drug regimens containing these inhibitors.

L20 ANSWER 21 OF 58 MEDLINE

2000085119 Document Number: 20085119. PubMed ID: 10618073. In vitro and in vivo evaluations of sodium lauryl sulfate and dextran sulfate as microbicides against herpes simplex and human immunodeficiency viruses. Piret J; Lamontagne J; Bestman-Smith J; Roy S; Gourde P; Desormeaux A; Omar R F; Juhasz J; Bergeron M G. (Centre de Recherche en Infectiologie, Universite Laval, Ste-Foy, Quebec, Canada G1V 4G2.) JOURNAL OF CLINICAL MICROBIOLOGY, (2000 Jan) 38 (1) 110-9. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB The efficacy of sodium lauryl sulfate (SLS), a sulfated anionic chaotropic surfactant, and dextran sulfate (DS), a polysulfated carbohydrate, against herpes simplex virus (HSV) and human immunodeficiency virus (HIV) infections was evaluated in cultured cells and in different murine models of HSV infection. Results showed that both SLS and DS were potent inhibitors of the infectivities of various HSV-1 and HSV-2 strains. Pretreatment of HIV-1 (strain NL4-3) with SLS also reduced its infectivity to 1G5 cells. DS prevented the binding of HSV to cell surface receptors and therefore its entry into cells. Pretreatment of HSV-1 (strain F) with 50 microM SLS resulted in a complete loss of virus infectivity to Vero cells. However, viruses were able to enter into cells and to produce in the nuclei capsid shells devoid of a DNA core. The amount of the glycoprotein D gene produced in these cells remained unchanged compared to controls, suggesting that SLS could interfere with the maturation of the virus. At a higher SLS concentration (100 microM), HSV was highly damaged by SLS pretreatment and only a few viral particles could enter into cells to produce abnormal capsids. Although DS was a more potent inhibitor of HSV infectivity in vitro, it was unable to provide any protection in murine models of HSV infection. However, SLS conferred a complete protection of animals infected cutaneously with pretreated viruses. In addition, skin pretreatment of mice with a polymer formulation containing SLS completely prevented the development of cutaneous lesions. More interestingly,

intravaginal pretreatment of mice with SLS in a buffered solution also completely protected against lethal HSV-2 infection. Taken together, our results suggest that SLS could thus represent a candidate of choice as a microbicide to prevent the sexual transmission of HIV, HSV, and possibly other pathogens that cause sexually transmitted diseases.

L20 ANSWER 29 OF 58 MEDLINE
1998197329 Document Number: 98197329. PubMed ID: 9536266.
Inhibition of HIV-1 replication by combined expression of gag dominant negative mutant and a human ribonuclease in a tightly controlled HIV-1 inducible vector. Cara A; Rybak S M; Newton D L; Crowley R; Rottschafer S E; Reitz M S Jr; Gusella G L. (Basic Research Laboratory, NCI, NIH, Bethesda, MD, USA.) GENE THERAPY, (1998 Jan) 5 (1) 65-75. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB An HIV-1-based expression vector has been constructed that produces protective genes tightly regulated by HIV-1 Tat and Rev proteins. The vector contains either a single protective gene (HIV-1 gag dominant negative mutant (delta-gag)) or a combination of two different protective genes (delta-gag and eosinophil-derived neurotoxin (EDN), a human ribonuclease) which are expressed from a dicistronic mRNA. After stable transfection of CEM T cells and following challenge with HIV-1, viral production was completely inhibited in cells transduced with the vector producing both delta-gag and EDN and delayed in cells producing delta-gag alone. In addition, cotransfection of HeLa-Tat cells with an infectious HIV-1 molecular clone and either protective vector demonstrated that the HIV-1 packaging signals present in the constructs were functional and allowed the efficient assembly of the protective RNAs into HIV-1 virions, thus potentially transmitting protection to the HIV-1 target cells.

L20 ANSWER 33 OF 58 MEDLINE
97239591 Document Number: 97239591. PubMed ID: 9085253.
Inhibition of early and late events of the HIV-1 replication cycle by cytoplasmic Fab intrabodies against the matrix protein, p17. Levin R; Mhashilkar A M; Dorfman T; Bukovsky A; Zani C; Bagley J; Hinkula J; Niedrig M; Albert J; Wahren B; Gottlinger H G; Marasco W A. (Division of Human Retrovirology, Dana-Farber Cancer Institute, Boston, MA 02115, USA.) MOLECULAR MEDICINE, (1997 Feb) 3 (2) 96-110. Journal code: 9501023. ISSN: 1076-1551. Pub. country: United States. Language: English.

AB BACKGROUND: The HIV-1 matrix (MA) protein, p17, contains two subcellular localization signals that facilitate both nuclear import of the viral preintegration complex early during infection and virus particle assembly late in infection. The dual role of MA in both the afferent and efferent arms of the HIV-1 life cycle makes it an important target for intracellular immunization-based gene therapy strategies. MATERIALS AND METHODS: Here we report, using a new bicistronic vector, that an intracellular Fab antibody, or Fab intrabody, directed against a carboxy-terminal epitope of MA from the Clade B HIV-1 genotype, can inhibit HIV-1 infection when expressed in the cytoplasm of actively dividing CD4+ T cells. RESULTS: Marked inhibition of proviral gene expression occurred when single-round HIV-1 CAT virus was used for infections. In challenge experiments using both laboratory strains and syncytium-inducing primary isolates of HIV-1, a substantial reduction in the infectivity of virions released from the cells was also observed. CONCLUSIONS: This novel strategy of

simultaneously blocking early and late events of the HIV-1 life cycle may prove useful in clinical gene therapy approaches for the treatment of HIV-1 infection and AIDS, particularly when combined with genetic or pharmacologic-based strategies that inhibit other HIV-1 target molecules simultaneously.

L20 ANSWER 40 OF 58 MEDLINE
96072970 Document Number: 96072970. PubMed ID: 7502043. Inhibitors of HIV nucleocapsid protein zinc fingers as candidates for the treatment of AIDS. Rice W G; Supko J G; Malspeis L; Buckheit R W Jr; Clanton D; Bu M; Graham L; Schaeffer C A; Turpin J A; Domagala J; +. (Laboratory of Antiviral Drug Mechanisms, PRI/DynCorp., National Cancer Institute-Frederick Cancer Research and Development Center, MD 21702, USA.) SCIENCE, (1995 Nov 17) 270 (5239) 1194-7. Journal code: 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB Strategies for the treatment of human immunodeficiency virus-type 1 (HIV-1) infection must contend with the obstacle of drug resistance. HIV-1 nucleocapsid protein zinc fingers are prime antiviral targets because they are mutationally intolerant and are required both for acute infection and virion assembly. Nontoxic disulfide-substituted benzamides were identified that attack the zinc fingers, inactivate cell-free virions, inhibit acute and chronic infections, and exhibit broad antiretroviral activity. The compounds were highly synergistic with other antiviral agents, and resistant mutants have not been detected. Zinc finger-reactive compounds may offer an anti-HIV strategy that restricts drug-resistance development.

L20 ANSWER 41 OF 58 MEDLINE
95266291 Document Number: 95266291. PubMed ID: 7747455. Inhibition of wild-type HIV-1 virus production by a matrix deficient Gag mutant. Lee P P; Linial M L. (Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104, USA.) VIROLOGY, (1995 Apr 20) 208 (2) 808-11. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Previous studies have shown that certain HIV-1 Gag mutants can interfere with the production of infectious HIV-1 when coexpressed with wild-type virus. In this paper, we studied two mutants of HIV-1 for their ability to interfere with the production of wild-type virus. Both mutants lack the entire matrix domain of gag and either lack [myr(-)MA (-)] or contain [myr(+)MA(-)] an amino-terminal myristate (myr) addition sequence at the beginning of the capsid domain. Previously we have demonstrated that expression of both mutant constructs leads to assembly and release of mutant viruses, although only myr(+)MA(-) particles are released efficiently. Particles produced by both matrix-deficient mutants are noninfectious and poorly incorporate and/or retain viral envelope glycoproteins. In this study, we further show that expression of myr(+)MA(-), but not myr(-)MA(-) interferes with wild-type HIV-1 virus production in transient expression assays. Our data suggest that wild-type and myristylated MA(-) Gag protein interacts at some point during assembly and that Gag myristylation has a greater effect on the assembly pathway than the matrix domain.

L21 ANSWER 14 OF 60 MEDLINE
2001133508 Document Number: 21066722. PubMed ID: 11145907. Effect of

mutations in Gag on assembly of immature human immunodeficiency virus type 1 capsids in a cell-free system. Singh A R; Hill R L; Lingappa J R. (Department of Physiology, University of California at San Francisco, San Francisco, California, 94117, USA.) VIROLOGY, (2001 Jan 5) 279 (1) 257-70. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Studies of HIV-1 capsid formation in a cell-free system revealed that capsid assembly occurs via an ordered series of assembly intermediates and requires host machinery. Here we use this system to examine 12 mutations in HIV-1 Gag that others studied previously in intact cells. With respect to capsid formation, these mutations generally produced the same phenotype in the cell-free system as in cells, indicating the cell-free system's high degree of fidelity. Analysis of assembly intermediates reveals that a mutation in the distal region of CA (322 LDeltaS) and truncations proximal to the second cys-his box in NC block multimerization of Gag at early stages in the cell-free capsid assembly pathway. In contrast, mutations in the region of amino acids 56-68 (located in the proximal portion of MA) inhibit assembly at a later point in the pathway. Other mutations, including truncations distal to the first cys-his box in NC and mutations in the distal half of MA (88HDeltaG, 85YDeltaG, Delta104-115, and Delta115-129), do not affect formation of immature capsids in the cell-free system. These data provide new information on the role of different domains in Gag during the early events of capsid assembly.

L21 ANSWER 26 OF 60 MEDLINE
1999092491 Document Number: 99092491. PubMed ID: 9875332. Gag protein from human immunodeficiency virus type 1 assembles in the absence of cyclophilin A. Streblow D N; Kitabwalla M; Pauza C D. (Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison 53706, USA.) VIROLOGY, (1998 Dec 5) 252 (1) 228-34. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Human immunodeficiency virus type 1 (HIV-1) replication requires coordinated activities of host and viral factors. We reported previously that interactions of the host factor cyclophilin A with HIV-1 Gag polyproteins affected Gag processing and maturation of virus particles (Streblow et al., 1998. Virology 245, 197-202). We now use in vitro translation and physical analysis of Gag structures to refine our understanding of how cyclophilin A affects HIV-1 replication. Gag assembled into oligomeric structures in vitro in the presence or absence of cyclophilin A, and proteins synthesized under the two conditions were equally susceptible to cleavage by exogenous HIV-1 protease. These and previous data show that Cyclophilin A is required at a step between Gag assembly and Gag processing/virion morphogenesis. Cyclophilin A may be required for Gag conformational changes subsequent to assembly, that are required for efficient dimerization and activation of the viral protease.

L21 ANSWER 33 OF 60 MEDLINE
1998169372 Document Number: 98169372. PubMed ID: 9501077. Proteolytic refolding of the HIV-1 capsid protein amino-terminus facilitates viral core assembly. von Schwedler U K; Stemmler T L; Klishko V Y; Li S; Albertine K H; Davis D R; Sundquist W I. (Department of

Biochemistry, University of Utah, Salt Lake City, UT 84132, USA.) EMBO JOURNAL, (1998 Mar 16) 17 (6) 1555-68. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB After budding, the human immunodeficiency virus (HIV) must 'mature' into an infectious viral particle. Viral maturation requires proteolytic processing of the Gag polyprotein at the matrix-capsid junction, which liberates the capsid (CA) domain to condense from the spherical protein coat of the immature virus into the conical core of the mature virus. We propose that upon proteolysis, the amino-terminal end of the capsid refolds into a beta-hairpin/helix structure that is stabilized by formation of a salt bridge between the processed amino-terminus (Pro1) and a highly conserved aspartate residue (Asp51). The refolded amino-terminus then creates a new CA-CA interface that is essential for assembling the condensed conical core. Consistent with this model, we found that recombinant capsid proteins with as few as four matrix residues fused to their amino-termini formed spheres in vitro, but that removing these residues refolded the capsid amino-terminus and redirected protein assembly from spheres to cylinders. Moreover, point mutations throughout the putative CA-CA interface blocked capsid assembly in vitro, core assembly in vivo and viral infectivity. Disruption of the conserved amino-terminal capsid salt bridge also abolished the infectivity of Moloney murine leukemia viral particles, suggesting that lenti- and oncoviruses mature via analogous pathways.

L21 ANSWER 40 OF 60 MEDLINE
97126066 Document Number: 97126066. PubMed ID: 8970990. The role of Gag in human immunodeficiency virus type 1 virion morphogenesis and early steps of the viral life cycle. Reicin A S; Ohagen A; Yin L; Hoglund S; Goff S P. (Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York 10032, USA.) JOURNAL OF VIROLOGY, (1996 Dec) 70 (12) 8645-52. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The phenotypes of a series of mutant human immunodeficiency virus type 1 proviruses with linker insertion and deletion mutations within the gag coding region were characterized. These mutants, with mutations in the matrix , capsid, and p2 coding regions, produced replication-defective virion particles with defects in the early steps of the viral life cycle. To investigate this phenotype further, the abilities of mutant virion particles to enter T cells, initiate and complete reverse transcription, and transport the newly transcribed proviral DNA were investigated. Only 4 of 10 of the mutants appeared to make wild-type levels of viral DNA. Biochemical analyses of the mutants revealed the middle region of CA as being important in determining virion particle density and sedimentation in velocity gradients. This region also appears to be critical in determining the morphology of mature virion particles by electron microscopy. Particles with aberrant morphology were uninfectious, and only those mutants which displayed cone-shaped cores were capable of carrying out the early steps of the viral life cycle. Thus, the normal morphology of human immunodeficiency virus type 1 appears to be critical to infectivity.

L21 ANSWER 43 OF 60 MEDLINE
96203962 Document Number: 96203962. PubMed ID: 8627263. Gag-Gag interactions in the C-terminal domain of human

immunodeficiency virus type 1 p24
capsid antigen are essential for Gag particle assembly.
Zhang W H; Hockley D J; Nermut M V; Morikawa Y; Jones I M. (Institute of Virology, Oxford, UK.) JOURNAL OF GENERAL VIROLOGY, (1996 Apr) 77 (Pt 4) 743-51. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Seven internal deletions within the p24 domain of the human immunodeficiency virus type 1 Gag precursor have been assessed for their effect on Gag particle formation following their expression using recombinant baculoviruses. In addition, each deleted molecule was assessed for its ability to bind soluble p24 antigen in vitro. The mutants fell into three different phenotypic groups: (i) three mutants that had no effect on either p24 binding or Gag particle assembly, (ii) three mutants that abolished both features and (iii) one mutant that bound p24 in vitro but failed to assemble particles. Mutations that abolished both in vitro p24 binding and particle assembly mapped to the C terminus of p24 confirming this region as critical for virion assembly. We suggest the division of virion assembly into at least two distinct phases and suggest a model in which the critical sequences mapped to date are combined with available structural information.

L23 ANSWER 22 OF 43 MEDLINE
95229748 Document Number: 95229748. PubMed ID: 7714031. A recombinant human immunodeficiency virus type-1 capsid protein (rp24): its expression, purification and physico-chemical characterization. Hausdorf G; Gewiess A; Wray V; Porstmann T. (Institut fur Biochemie der Charite Humboldt-Universitat, Medizinische Fakultat (Charite), Med. Universit atsklinik III, Berlin, Germany.) JOURNAL OF VIROLOGICAL METHODS, (1994 Dec) 50 (1-3) 1-9. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB An expression system has been established in Escherichia coli to facilitate the preparation of the HIV-1 capsid protein in amounts sufficient for structural analysis. A plasmid vector pTCA5, containing the gene for the recombinant HIV-1 capsid protein rp24 under the control of the lambda-PR-promoter, was constructed which gave an expression product that spanned 234 amino acid residues. It differs at the N-terminus from the authentic sequence in that the residues Pro-Ile- are replaced by Met-Asn-Ser-Ala-Met-. Recombinant p24 was produced, as inclusion bodies in E. coli LE392 containing pTCA5, at a level of approximately 15% of the total cellular protein. After dissolution of the inclusion bodies in the acidic urea system, the protein was easily reconstituted in a soluble state by dialysis. The yield of reconstituted and purified protein was 12 mg per liter in rich medium. Recombinant rp24 consists of about 40% alpha-helix and 10% beta-sheet from circular dichroism measurements and the two cysteine residues, within the rp24 sequence, are bridged by a disulfide bond.

L23 ANSWER 31 OF 43 MEDLINE
93091540 Document Number: 93091540. PubMed ID: 1458053. Bacterial expression, purification, and in vitro N-myristoylation of HIV-1 p17gag. Burnette B; Kahn R; Glover C J; Felsted R L. (Laboratory of Biological Chemistry, National Cancer Institute, Bethesda, Maryland 20894.) PROTEIN EXPRESSION AND PURIFICATION, (1992 Oct) 3 (5) 395-402. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB The coding region of the N-terminal 17-kDa portion of HIV-1 Pr55gag (p17gag) was cloned into the pET-3c expression vector and was used to overexpress HIV-1 p17gag in Escherichia coli. Induction of the transformed bacteria caused the accumulation of a 17-kDa polypeptide in the soluble cell fraction which was released by sonication in hypotonic nondetergent buffer. The 17-kDa polypeptide was purified by ammonium sulfate precipitation and successive chromatography on G-75 Sephadex, DEAE-Sephacel, and S-Sephadex. The final product was purified 12-fold with about a 16% recovery from the original soluble cell lysate and was judged to be 97+% pure by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Western blotting with two different antibodies confirmed the identify of the purified 17-kDa polypeptide as authentic p17gag. In the presence of myristoyl-CoA and bovine brain N-myristoyl-transferase, p17gag was quantitatively N-myristoylated in vitro with a pseudo-first-order rate constant of 4.7 +/- 1.0 x 10(-3) min-1, but with only about 3% of the catalytic efficiency of N-myristoylation of a 16-residue peptide homologous to the N-terminus of p17gag. The myristate group in the N-myristoylated p17gag was stable to treatment with detergent and hydroxylamine consistent with a covalent N-acyl-amide linkage. The N-myristoylglycyl linkage was confirmed by partial acid hydrolysis and identification of the p-nitrobenzylazlactone derivative of the resulting N-myristoylglycine by high-performance liquid chromatography.

L23 ANSWER 37 OF 43 MEDLINE
91128665 Document Number: 91128665. PubMed ID: 2126440. Expression and purification of p24, the core protein of HIV, using a baculovirus-insect cell expression system. Mills H R; Jones I M. (Natural Environment Research Council (NERC), Institute of Virology and Environmental Microbiology, Oxford, UK.) AIDS, (1990 Nov) 4 (11) 1125-31. Journal code: 8710219. ISSN: 0269-9370. Pub. country: United States. Language: English.

AB The baculovirus Autographa californica nuclear polyhedrosis virus (AcNPV) has been genetically manipulated to yield a recombinant virus capable of expressing p24, the major core protein of HIV-1, in insect cell culture. The expressed product is a p24 protein flanked by short regions of p17 at the amino terminus and p12 at the carboxy terminus. It has been identified and characterized using monoclonal antibodies on Western blots and by amino-terminal sequence analysis. The presence of p24 in the soluble fraction of infected cells following lysis by detergent or sonication, combined with a high level of expression (in excess of 50 mg/l of culture) facilitates the enrichment of large quantities of recombinant HIV antigen in a simple two-step procedure involving ammonium sulphate fractionation and gel filtration. p24 antigen purified in this way is shown to be an efficient diagnostic reagent.

L23 ANSWER 43 OF 43 MEDLINE
89308620 Document Number: 89308620. PubMed ID: 2663848. Purification and characterization of human immunodeficiency virus (HIV) core precursor (p55) expressed in Saccharomyces cerevisiae. Vlasuk G P; Waxman L; Davis L J; Dixon R A; Schultz L D; Hofmann K J; Tung J S; Schulman C A; Ellis R W; Bencen G H; +. (Department of Biological Chemistry, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Jul 15) 264 (20) 12106-12. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The core structure of retroviruses, including the human immunodeficiency virus (HIV), consists of

proteins that are initially synthesized as polyprotein precursors and then processed by a virally encoded protease yielding the mature core polypeptides. To obtain sufficient quantities of the purified HIV core precursor p55 for detailed studies, a segment of HIV DNA encoding the full length core precursor polyprotein p55 was expressed in Saccharomyces cerevisiae using a plasmid containing a constitutive galactose promoter. The expression of this DNA produced a protein with an estimated molecular size of 55,000, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE); this protein was immunoreactive to anti-HIV p24 antisera. Following cell lysis, freezing, and thawing, the expressed protein was an insoluble aggregate that served as the starting material for the purification process. **Solubilization of the insoluble p55 with guanidine HCl followed by phenyl-Sepharose column chromatography and high performance liquid chromatography resulted in a preparation of p55 that was greater than 95% pure by SDS-PAGE, immunoreactive to anti-HIV core protein antibodies, and completely soluble in aqueous solution.** The expressed p55 appeared to be myristoylated as evidenced by the incorporation of radiolabel following incubation of recombinant yeast cells with [3H]myristic acid; in addition the amino terminus of the final purified protein was blocked. Proteolytic digestion of purified p55 with synthetic HIV protease yielded the predicted amino- and carboxyl-terminal products; these were confirmed by amino acid sequence analysis. In contrast, digestion of purified p55 by the protease derived from the avian myeloblastosis virus resulted in fragments that were different in size from those produced by the HIV protease. The availability of the purified, full length water-soluble HIV core precursor will be useful in identifying agents that inhibit its processing by the HIV protease.

L27 ANSWER 46 OF 98 MEDLINE
1999139010 Document Number: 99139010. PubMed ID: 9971810. In vitro assembly properties of human immunodeficiency virus type 1 Gag protein lacking the p6 domain. Campbell S; Rein A. (ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702, USA.. campbells@mail.ncifcrf.gov) . JOURNAL OF VIROLOGY, (1999 Mar) 73 (3) 2270-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Human immunodeficiency virus type 1 (HIV-1) normally assembles into particles of 100 to 120 nm in diameter by budding through the plasma membrane of the cell. The Gag polyprotein is the only viral protein that is required for the formation of these particles. We have used an in vitro assembly system to examine the assembly properties of purified, recombinant HIV-1 Gag protein and of Gag missing the C-terminal p6 domain (Gag Deltap6). This system was used previously to show that the CA-NC fragment of HIV-1 Gag assembled into cylindrical particles. We now report that both HIV-1 Gag and Gag Deltap6 assemble into small, 25- to 30-nm-diameter spherical particles in vitro. The multimerization of Gag Deltap6 into units larger than dimers and the formation of spherical particles required nucleic acid. Removal of the nucleic acid with NaCl or nucleases resulted in the disruption of the multimerized complexes. We conclude from these results that (i) N-terminal extension of HIV -1 CA-NC to include the MA domain results in the formation of spherical, rather than cylindrical, particles; (ii) nucleic acid is required for the assembly and maintenance of

HIV-1 Gag Deltap6 virus-like particles in vitro and possibly in vivo; (iii) a wide variety of RNAs or even short DNA oligonucleotides will support assembly; (iv) protein-protein interactions within the particle must be relatively weak; and (v) recombinant HIV-1 Gag Deltap6 and nucleic acid are not sufficient for the formation of normal-sized particles.

L27 ANSWER 49 OF 98 MEDLINE
1999102696 Document Number: 99102696. PubMed ID: 9872746. Assembly and analysis of conical models for the HIV-1 core. Ganser B K; Li S; Klishko V Y; Finch J T; Sundquist W I. (Department of Biochemistry, University of Utah, Salt Lake City, UT 84132, USA.) SCIENCE, (1999 Jan 1) 283 (5398) 80-3. Journal code: 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB The genome of the human immunodeficiency virus (HIV) is packaged within an unusual conical core particle located at the center of the infectious virion. The core is composed of a complex of the NC (nucleocapsid) protein and genomic RNA, surrounded by a shell of the CA (capsid) protein. A method was developed for assembling cones in vitro using pure recombinant HIV-1 CA-NC fusion proteins and RNA templates. These synthetic cores are capped at both ends and appear similar in size and morphology to authentic viral cores. It is proposed that both viral and synthetic cores are organized on conical hexagonal lattices, which by Euler's theorem requires quantization of their cone angles. Electron microscopic analyses revealed that the cone angles of synthetic cores were indeed quantized into the five allowed angles. The viral core and most synthetic cones exhibited cone angles of approximately 19 degrees (the narrowest of the allowed angles). These observations suggest that the core of HIV is organized on the principles of a fullerene cone, in analogy to structures recently observed for elemental carbon.

L27 ANSWER 51 OF 98 MEDLINE
1998241716 Document Number: 98241716. PubMed ID: 9573245. N-Terminal extension of human immunodeficiency virus capsid protein converts the in vitro assembly phenotype from tubular to spherical particles. Gross I; Hohenberg H; Huckhagel C; Krausslich H G. (Heinrich-Pette-Institut, D-20251 Hamburg, Germany.) JOURNAL OF VIROLOGY, (1998 Jun) 72 (6) 4798-810. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Expression of retroviral Gag polyproteins is sufficient for morphogenesis of virus-like particles with a spherical immature protein shell. Proteolytic cleavage of Gag into the matrix (MA), capsid (CA), nucleocapsid (NC), and p6 domains (in the case of human immunodeficiency virus [HIV]) leads to condensation to the mature cone-shaped core. We have analyzed the formation of spherical or cylindrical particles on in vitro assembly of purified HIV proteins or inside Escherichia coli cells. CA protein alone yielded cylindrical particles, while all N-terminal extensions of CA abolished cylinder formation. Spherical particles with heterogeneous diameters or amorphous protein aggregates were observed instead. Extending CA by 5 amino acids was sufficient to convert the assembly phenotype to spherical particles. Sequences C-terminal of CA were not required for sphere formation. Proteolytic cleavage of N-terminally extended CA proteins prior to in vitro assembly led to the formation of cylindrical particles, while

proteolysis of in vitro assembly products caused disruption of spheres but not formation of cylinders. In vitro assembly of CA and extended CA proteins in the presence of cyclophilin A (CypA) at a CA-to-CypA molar ratio of 10:1 yielded significantly longer cylinders and heterogeneous spheres, while higher concentrations of CypA completely disrupted particle formation. We conclude that the spherical shape of immature HIV particles is determined by the presence of an N-terminal extension on the CA domain and that core condensation during virion maturation requires the liberation of the N terminus of CA.

L27 ANSWER 54 OF 98 MEDLINE
1998036138 Document Number: 98036138. PubMed ID: 9370371. In vitro assembly properties of purified bacterially expressed capsid proteins of human immunodeficiency virus. Gross I; Hohenberg H; Krausslich H G. (Heinrich-Pette-Institut, Hamburg, Germany.) EUROPEAN JOURNAL OF BIOCHEMISTRY, (1997 Oct 15) 249 (2) 592-600. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The Gag polyprotein of retroviruses is sufficient for assembly and budding of virus-like particles from the host cell. In the case of human immunodeficiency virus (HIV), Gag contains the domains matrix, capsid (CA), nucleocapsid (NC) and p6 which are separated by the viral proteinase inside the nascent virion, leading to morphological maturation to yield an infectious virus. In the mature virus, CA forms a capsid shell surrounding the ribonucleoprotein core consisting of NC and the genomic RNA. To define requirements for particle assembly and functional contributions of individual domains, we expressed domains of HIV Gag in Escherichia coli and purified the products to near homogeneity. In vitro assembly of CA, with or without the C-terminally adjacent spacer peptide, yielded tubular structures with a diameter of approximately 55 nm and heterogeneous length. **Efficient particle formation required high protein concentration, high salt and neutral to alkaline pH.** In contrast, **in vitro assembly of CA-NC occurred at a 20-fold lower protein concentration and in low salt, but required addition of RNA.** These results suggest that hydrophobic interactions of capsid proteins are sufficient for particle formation while the RNA-binding nucleocapsid domain may concentrate and align structural proteins on the viral genome.

L27 ANSWER 63 OF 98 MEDLINE
97048110 Document Number: 97048110. PubMed ID: 8892951. Human immunodeficiency virus type 1 capsid formation in reticulocyte lysates. Spearman P; Ratner L. (Department of Pediatrics, Vanderbilt University, Nashville, Tennessee 37232-2581, USA.) JOURNAL OF VIROLOGY, (1996 Nov) 70 (11) 8187-94. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The Gag polyprotein of human immunodeficiency virus (HIV) (Pr55Gag) contains sufficient information to direct particle assembly events when expressed within tissue culture cells. HIV Gag proteins normally form particles at a plasma membrane assembly site, in a manner analogous to that of the type C avian and mammalian leukemia/sarcoma viruses. It has not previously been demonstrated that

immature HIV capsids can form without budding through an intact cellular membrane. In this study, a rabbit reticulocyte lysate translation reaction was used to recreate HIV capsid formation in vitro. Production of HIV-1 Pr55Gag and of a matrix-deleted Gag construct resulted in the formation of a subset of Gag protein structures with an equilibrium density of 1.15 g/ml. Gel filtration chromatography revealed these Gag protein structures to be larger than 2 x 10(6) Da, consistent with the formation of large multimers or capsids. These Gag protein structures were protease sensitive in the absence of detergent, indicating that they did not contain a complete lipid envelope. Spherical structures were detected by electron microscopy within the reticulocyte lysate reaction mixtures and appeared essentially identical to immature HIV capsids or retrovirus-like particles. These results demonstrate that the HIV Gag protein is capable of producing immature capsids in a cell-free reaction and that such capsids lack a complete lipid envelope.

L27 ANSWER 65 OF 98 MEDLINE
96211503 Document Number: 96211503. PubMed ID: 8648705. Synthesis and assembly of retrovirus Gag precursors into immature capsids in vitro. Sakalian M; Parker S D; Weldon R A Jr; Hunter E. (Department of Microbiology, University of Alabama at Birmingham, 35294-2170, USA.) JOURNAL OF VIROLOGY, (1996 Jun) 70 (6) 3706-15. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The assembly of retroviral particles is mediated by the product of the gag gene; no other retroviral gene products are necessary for this process. While most retroviruses assemble their capsids at the plasma membrane, viruses of the type D class preassemble immature capsids within the cytoplasm of infected cells. This has allowed us to determine whether immature capsids of the prototypical type D retrovirus, Mason-Pfizer monkey virus (M-PMV), can assemble in a cell-free protein synthesis system. We report here that assembly of M-PMV Gag precursor proteins can occur in this in vitro system. Synthesized particles sediment in isopycnic gradients to the appropriate density and in thin-section electron micrographs have a size and appearance consistent with those of immature retrovirus capsids. The in vitro system described in this report appears to faithfully mimic the process of assembly which occurs in the host cell cytoplasm, since M-PMV gag mutants defective in in vivo assembly also fail to assemble in vitro. Likewise, the Gag precursor proteins of retroviruses that undergo type C morphogenesis, Rous sarcoma virus and human immunodeficiency virus, which do not preassemble capsids in vivo, fail to assemble particles in this system. Additionally, we demonstrate, with the use of anti-Gag antibodies, that this cell-free system can be utilized for analysis in vitro of potential inhibitors of retrovirus assembly.

L27 ANSWER 70 OF 98 MEDLINE
95395987 Document Number: 95395987. PubMed ID: 7666550. Self-assembly in vitro of purified CA-NC proteins from Rous sarcoma virus and human immunodeficiency virus type 1. Campbell S; Vogt V M. (Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853, USA.) JOURNAL OF VIROLOGY, (1995 Oct) 69 (10) 6487-97. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The internal structural proteins of retroviruses are proteolytically processed from the Gag polyprotein, which alone is able to assemble into virus-like particles when expressed in cells. All Gag proteins contain domains corresponding to the three structural proteins MA, CA, and NC. We have expressed the CA and NC domains together as a unit in Escherichia coli, both for Rous sarcoma virus (RSV) and for human immunodeficiency virus type 1 (HIV-1). We also expressed a similar HIV-1 protein carrying the C-terminal p6 domain. RSV CA-NC, HIV-1 CA-NC, and HIV-1 CA-NC-p6 were purified in native form by classic methods. **After adjustment of the pH and salt concentration, each of these proteins was found to assemble at a low level of efficiency into structures that resembled circular sheets and roughly spherical particles.** The presence of RNA dramatically increased the efficiency of assembly, and in this case all three proteins formed hollow, cylindrical particles whose lengths were determined by the size of the RNA. **The optimal pH at which assembly occurred was 5.5 for the RSV protein and 8.0 for the HIV-1 proteins.** The treatment of the RSV CA-NC cylindrical particles with nonionic detergent, with ribonuclease, or with viral protease caused disassembly. These results suggest that RNA plays an important structural role in the virion and that it may initiate and organize the assembly process. The in vitro system described should facilitate the dissection of assembly pathways in retroviruses.

L27 ANSWER 76 OF 98 MEDLINE
94267433 Document Number: 94267433. PubMed ID: 8207412. Inhibition of infectious human immunodeficiency virus type 1 particle formation by Gag protein-derived peptides. Niedrig M; Gelderblom H R; Pauli G; Marz J; Bickhard H; Wolf H; Modrow S. (Behringwerke AG, Marburg, Germany.) JOURNAL OF GENERAL VIROLOGY, (1994 Jun) 75 (Pt 6) 1469-74. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Sequential overlapping Gag protein-derived oligopeptides of human immunodeficiency virus type 1 (HIV-1) 22 to 24 amino acids long, were synthesized and tested in vitro for antiviral activity. Two synthetic peptides, one derived from the matrix protein p17 (NPGLLETSEGCRQ, amino acids 47 to 59) and one located in the capsid protein p24 (PAATLEEMMTA, amino acids 339 to 349) inhibited the production of infectious virus when added to HIV-1-infected cultures when used in the range of 20 to 200 micrograms/ml. As shown by thin section electron microscopy, peptide treatment resulted in the release of immature, deformed virus particles suggesting that the two peptides interfered with assembly and maturation. Other Gag protein-derived oligopeptides had little or no influence on virus production. To characterize further the functionally active regions we synthesized peptide derivatives with three consecutive amino acids substituted by alanine; they did not cause inhibition. Therefore the regions responsible for inhibition were located between amino acids 50 to 61 in p17, and 342 to 350 in p24. These observations might lead to the development of a new antiviral strategy affecting the late stage of virus replication.

L27 ANSWER 77 OF 98 MEDLINE
94240182 Document Number: 94240182. PubMed ID: 8183954. Characterization of human immunodeficiency virus type 1 Pr55gag membrane association in a cell-free system: requirement

for a C-terminal domain. Platt E J; Haffar O K. (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 May 10) 91 (10) 4594-8. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Association of the human immunodeficiency virus type 1 (HIV-1) gag polyprotein precursor with cellular membranes is necessary for assembly of virions. We used in vitro synthesized HIV-1 gag to study its association with isolated cellular membranes. Rabbit reticulocyte lysates programmed with HIV-1 gag mRNA incorporated [35S]methionine and [3H]myristate into two predominant species of 55 kDa and 40 kDa. Radioimmunoprecipitation with HIV -1-specific antibodies suggested that the 55-kDa protein represented the polyprotein precursor (Pr55gag), while the 40-kDa protein was a mixture of N- or C-terminal truncations of the gag precursor. The Pr55gag protein bound to cellular membranes, while the 40-kDa mixed protein species did not. Membrane binding studies with C terminus-truncated and point mutants revealed that the seven-amino acid sequence located between the two Cys-His arrays in the nucleocapsid region was necessary for stable association to occur. Therefore, we propose that signals in addition to myristate are required for the membrane association of HIV-1 gag proteins and that these signals include a domain in the nucleocapsid protein.

L27 ANSWER 83 OF 98 MEDLINE
93287246 Document Number: 93287246. PubMed ID: 7685414. Assembly , processing, and infectivity of human immunodeficiency virus type 1 gag mutants. Wang C T; Barklis E. (Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland 97201.) JOURNAL OF VIROLOGY, (1993 Jul) 67 (7) 4264-73. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We studied the effects of gag mutations on human immunodeficiency virus type 1 (HIV-1) assembly, processing, and infectivity by using a replication-defective HIV expression system. HIV mutants were screened for infectivity by transduction of a selectable marker and were examined for assembly by monitoring particle release from transfected cells. Gag protein processing and reverse transcriptase activities of mutant particles were also assayed. Surprisingly, most Gag protein mutants were assembled and processed. The two exceptions to this rule were a myristylation-minus mutant, and one gag matrix domain mutant which expressed proteins that were trapped intracellularly. Interestingly, a mutant with a 56-amino-acid deletion within the HIV gag capsid domain still could assemble and process virus particles, exhibited a wild-type retrovirus particle density, and had wild-type reverse transcriptase activity. Indeed, although most HIV-1 gag mutants were noninfectious or poorly infectious, they produced apparently normal particles which possessed significant reverse transcriptase activities. These results strongly support the notion that the HIV-1 Gag proteins are functionally involved in post-assembly, postprocessing stages of virus infectivity.

L27 ANSWER 85 OF 98 MEDLINE
93188179 Document Number: 93188179. PubMed ID: 8445731. Requirements for incorporation of Pr160gag-pol from human immunodeficiency virus type 1 into virus-like particles.

Smith A J; Srinivasakumar N; Hammarskjold M L; Rekosh D. (Department of Microbiology, State University of New York, Buffalo 14214.) JOURNAL OF VIROLOGY, (1993 Apr) 67 (4) 2266-75. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The roles of the human immunodeficiency virus precursor polyproteins Pr55gag and Pr160gag-pol in viral core assembly were studied in CMT3-COS cells. To do this, the precursors were expressed separately by using a simian virus 40 late replacement vector system described previously. Consistent with previously published data, our results show that the Pr55gag precursor, when expressed alone, was able to form particles which had an immature morphology and that particle formation required the presence of a myristate addition signal at the amino terminus of the precursor. In contrast, the Pr160gag-pol precursor was not able to form particles when expressed alone, although it still underwent proteolytic processing. Coexpression of the two precursor polyproteins from separate vectors in the same cell resulted in processing of the Pr55gag in trans by the protease embedded in Pr160gag-pol and the formation of virus-like particles containing the products of both precursors. Proteolytic processing occurred independently of the presence of a functional myristate addition signal on either precursor. On the other hand, removal of myristate from one or the other precursor had nonreciprocal effects on virus particle formation. Cells expressing Pr55gag lacking myristate and Pr160gag-pol containing it did not produce particles. Cells expressing a myristylated Pr55gag and unmyristylated Pr160gag-pol still produced virus-like particles which contained nearly normal amounts of Pr160gag-pol. The results suggest that the incorporation of Pr160gag-pol into particles is largely determined by intermolecular protein-protein interactions between the two precursor polypeptides.

L27 ANSWER 88 OF 98 MEDLINE
92333669 Document Number: 92333669. PubMed ID: 1629958. Assembly of recombinant human immunodeficiency virus type 1 capsid protein in vitro. Ehrlich L S; Agresta B E; Carter C A. (Department of Microbiology, State University of New York, Stony Brook 11794.) JOURNAL OF VIROLOGY, (1992 Aug) 66 (8) 4874-83. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The capsid protein (CA) (p24) of human immunodeficiency virus (HIV) type 1 expressed in Escherichia coli and purified to greater than 90% homogeneity was used to examine assembly in vitro and to probe the nature of interactions involved in the formation of capsid structures. The protein was detected in dimeric and oligomeric forms as indicated by molecular size measurements by gel filtration column chromatography, sedimentation through sucrose, and nondenaturing gel electrophoresis. Chemical cross-linking of CA molecules was observed with several homobifunctional reagents. Oligomer size was dependent on cross-linker concentration and exhibited a nonrandom pattern in which dimers and tetramers were more abundant than trimers and pentamers. Oligomers as large as dodecamers were detected in native polyacrylamide gels. **These were stable in solutions of high ionic strength or in the presence of nonionic detergent, indicating that strong interactions were involved in oligomer stabilization.** Limited tryptic digestion converted the putative dodecamers to octamers, suggesting that a region involved in CA protein multimerization was exposed in the structure. This region was mapped to the central portion of the protein.

The recombinant CA proteins assembled in vitro into long rodlike structures and were disassembled into small irregular spheres by alterations in ionic strength and pH. The observation that assembly and disassembly of purified HIV type 1 CA protein can be induced in vitro suggests an approach for identifying possible control mechanisms involved in HIV viral core assembly.

L27 ANSWER 35 OF 98 MEDLINE
2000094903 Document Number: 20094903. PubMed ID: 10627527. Biochemical and structural analysis of isolated mature cores of human immunodeficiency virus type 1. Welker R; Hohenberg H; Tessmer U; Huckhagel C; Krausslich H G. (Heinrich-Pette-Institut fur experimentelle Virologie und Immunologie an der Universitat Hamburg, D-20251 Hamburg, Germany.) JOURNAL OF VIROLOGY, (2000 Feb) 74 (3) 1168-77. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Mature human immunodeficiency virus type 1 (HIV-1) particles contain a cone-shaped core structure consisting of the internal ribonucleoprotein complex encased in a proteinaceous shell derived from the viral capsid protein. Because of their very low stability after membrane removal, HIV-1 cores have not been purified in quantities sufficient for structural and biochemical analysis. Based on our **in vitro assembly experiments**, we have developed a novel method for isolation of intact mature HIV-1 cores. **Concentrated virus suspensions were briefly treated with nonionic detergent and immediately centrifuged in a microcentrifuge for short periods of time.** The resuspended pellet was subsequently analyzed by negative-stain and thin-section electron microscopy and by immunoelectron microscopy. Abundant cone-shaped cores as well as tubular and aberrant structures were observed. Stereo images showed that core structures preserved their three-dimensional architecture and exhibited a regular substructure. Detailed analysis of 155 cores revealed an average length of ca. 103 nm, an average diameter at the base of ca. 52 nm, and an average angle of 21.3 degrees. There was significant variability in all parameters, indicating that HIV cores are not homogeneous. Immunoblot analysis of core preparations allowed semiquantitative estimation of the relative amounts of viral and cellular proteins inside the HIV-1 core, yielding a model for the topology of various proteins inside the virion.

L27 ANSWER 41 OF 98 MEDLINE
1999225664 Document Number: 99225664. PubMed ID: 10208938. In vitro assembly properties of wild-type and cyclophilin-binding defective human immunodeficiency virus capsid proteins in the presence and absence of cyclophilin A. Grattinger M; Hohenberg H; Thomas D; Wilk T; Muller B; Krausslich H G. (Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie an der, Universitat Hamburg, Hamburg, D-20251, Germany.) VIROLOGY, (1999 Apr 25) 257 (1) 247-60. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The cellular protein cyclophilin A (CypA) binds specifically to the human immunodeficiency virus type 1 (HIV-1) capsid (CA) protein and is incorporated into HIV-1 particles at a molar ratio of 1:10 (CypA/CA). Structural analysis of a CA-CypA complex suggested that CypA may destabilize interactions in the viral capsid and thus promote uncoating. We analyzed the influence of CypA on the in

vitro assembly properties of wild-type (WT) CA and derivatives containing substitutions of Gly89 in the Cyp-binding loop. All variant proteins were significantly impaired in CypA binding. In the presence of CypA at a molar ratio of 1:10 (CypA/CA), WT CA assembled into hollow cylinders that were similar to those observed in the absence of CypA but slightly longer. Higher CypA concentrations inhibited cylinder formation. Variant CA proteins G89L and G89F yielded similar cylinders as the WT protein but were significantly more resistant to CypA. Cryoelectron microscopic analysis of WT cylinders assembled in the presence of CypA revealed direct binding of CypA to the outer surface. Electron diffraction patterns generated from these cylinders indicated that CypA causes local disorder. The addition of CypA to preassembled cylinders had little effect, however, and cylinders were only disrupted when incubated with a threefold molar excess of CypA for several hours. These results suggest that CypA does not efficiently destabilize CA interactions at the molar ratio observed in the virion and therefore is unlikely to serve as an uncoating factor.

L27 ANSWER 39 OF 98 MEDLINE
1999292859 Document Number: 99292859. PubMed ID: 10364315. Formation of virus assembly intermediate complexes in the cytoplasm by wild-type and assembly-defective mutant human immunodeficiency virus type 1 and their association with membranes. Lee Y M; Liu B; Yu X F. (Department of Molecular Microbiology and Immunology, Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland 21205, USA.) JOURNAL OF VIROLOGY, (1999 Jul) 73 (7) 5654-62. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We have previously identified two distinct forms of putative viral assembly intermediate complexes, a detergent-resistant complex (DRC) and a detergent-sensitive complex (DSC), in human immunodeficiency virus type 1 (HIV-1)-infected CD4(+) T cells (Y. M. Lee and X. F. Yu, Virology 243:78-93, 1998). In the present study, the intracellular localization of these two viral assembly intermediate complexes was investigated by use of a newly developed method of subcellular fractionation. In wild-type HIV -1-infected H9 cells, the DRC fractionated with the soluble cytoplasmic fraction, whereas the DSC was associated with the membrane fraction. The DRC was also detected in the cytoplasmic fraction in H9 cells expressing HIV-1 Myr- mutant Gag. However, little of the unmyristylated Gag and Gag-Pol proteins was found in the membrane fraction. Furthermore, HIV-1 Gag proteins synthesized in vitro in a rabbit reticulocyte lysate system in the absence of exogenous lipid membrane were able to assemble into a viral Gag complex similar to that of the DRC identified in infected H9 cells. The density of the viral Gag complex was not altered by treatment with the nonionic detergent Triton X-100, suggesting a lack of association of this complex with endogenous lipid. Formation of the DRC was not significantly affected by mutations in assembly domains M and L of the Gag protein but was drastically inhibited by a mutation in the assembly I domain. Purified DRC could be disrupted by high-salt treatment, suggesting electrostatic interactions are important for stabilizing the DRC. The Gag precursor proteins in the DRC were more sensitive to trypsin digestion than those in the DSC. These findings suggest that HIV -1 Gag and Gag-Pol precursors assemble into DRC in the cytoplasm, a process which requires the protein-protein interaction domain (I) in NCp7; subsequently, the DRC is transported to the plasma membrane

through a process mediated by the M domain of the matrix protein. It appears that during this process, a conformational change might occur in the DRC either before or after its association with the plasma membrane, and this change is followed by the detection of virus budding structure at the plasma membrane.

L27 ANSWER 38 OF 98 MEDLINE
1999419092 Document Number: 99419092. PubMed ID: 10488150. In vitro assembly of human immunodeficiency virus type 1 Gag protein.
Morikawa Y; Goto T; Sano K. (The Kitasato Institute, Shirokane 5-9-1, Minato-ku, Tokyo 108-8642, Japan.. ymorikawa@kitasato.or.jp) . JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Sep 24) 274 (39) 27997-8002. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Retroviral Gag protein is sufficient to produce Gag virus-like particles when expressed in higher eukaryotic cells. Here we describe the in vitro assembly reaction of human immunodeficiency virus Gag protein, which consists of two sequential steps showing the optimal conditions for each reaction. Following expression and purification, Gag protein lacking only the C-terminal p6 domain was present as a monomer (50 kDa) by velocity sedimentation analysis. **Initial assembly of the Gag protein to 60 S intermediates occurred by dialysis at 4 degrees C in low salt at neutral to alkaline pH.** However, **higher order of assembly required incubation at 37 degrees C and was facilitated by the addition of Mg(2+).** Prolonged incubation under these conditions produced complete assembly (600 S), equivalent to Gag virus-like particles obtained from Gag-expressing cells. **Neither form disassembled by treatment with nonionic detergent,** suggesting that correct assembly might occur in vitro. Electron microscopic observation confirmed that the 600 S assembly products were spherical particles similar to authentic immature human immunodeficiency virus particles. The latter assembly stage but not the former was accelerated by the addition of RNA although not inhibited by RNaseA treatment. These results suggest that Gag protein alone assembles in vitro, but that additional RNA facilitates the assembly reaction.

L27 ANSWER 36 OF 98 MEDLINE
2000086825 Document Number: 20086825. PubMed ID: 10619849. A conformational switch controlling HIV-1 morphogenesis. Gross I; Hohenberg H; Wilk T; Wiegers K; Grattinger M; Muller B; Fuller S; Krausslich H G. (Heinrich-Pette-Institut fur experimentelle Virologie und Immunologie an der Universitat Hamburg, Martinistrasse 52, D-20251 Hamburg, Germany.) EMBO JOURNAL, (2000 Jan 4) 19 (1) 103-13. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Assembly of infectious human immunodeficiency virus type 1 (HIV-1) proceeds in two steps. Initially, an immature virus with a spherical capsid shell consisting of uncleaved Gag polyproteins is formed. Extracellular proteolytic maturation causes rearrangement of the inner virion structure, leading to the conical capsid of the infectious virus. Using an **in vitro assembly system,** we show that the same HIV -1 Gag-derived protein can form spherical particles, virtually indistinguishable from immature HIV-1 capsids, as well as tubular or conical particles, resembling the mature core. The

assembly phenotype could be correlated with differential binding of the protein to monoclonal antibodies recognizing epitopes in the HIV-1 capsid protein (CA), suggesting distinct conformations of this domain. Only tubular and conical particles were observed when the protein lacked spacer peptide SP1 at the C-terminus of CA, indicating that SP1 may act as a molecular switch, whose presence determines spherical capsid formation, while its cleavage leads to maturation.

L27 ANSWER 35 OF 98 MEDLINE
2000094903 Document Number: 20094903. PubMed ID: 10627527. Biochemical and structural analysis of isolated mature cores of human immunodeficiency virus type 1. Welker R; Hohenberg H; Tessmer U; Huckhagel C; Krausslich H G. (Heinrich-Pette-Institut fur experimentelle Virologie und Immunologie an der Universitat Hamburg, D-20251 Hamburg, Germany.) JOURNAL OF VIROLOGY, (2000 Feb) 74 (3) 1168-77. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Mature human immunodeficiency virus type 1 (HIV-1) particles contain a cone-shaped core structure consisting of the internal ribonucleoprotein complex encased in a proteinaceous shell derived from the viral capsid protein. Because of their very low stability after membrane removal, HIV-1 cores have not been purified in quantities sufficient for structural and biochemical analysis. Based on our in vitro assembly experiments, we have developed a novel method for isolation of intact mature HIV-1 cores. **Concentrated virus suspensions were briefly treated with nonionic detergent and immediately centrifuged in a microcentrifuge for short periods of time.** The resuspended pellet was subsequently analyzed by negative-stain and thin-section electron microscopy and by immunoelectron microscopy. Abundant cone-shaped cores as well as tubular and aberrant structures were observed. Stereo images showed that core structures preserved their three-dimensional architecture and exhibited a regular substructure. Detailed analysis of 155 cores revealed an average length of ca. 103 nm, an average diameter at the base of ca. 52 nm, and an average angle of 21.3 degrees. There was significant variability in all parameters, indicating that HIV cores are not homogeneous. Immunoblot analysis of core preparations allowed semiquantitative estimation of the relative amounts of viral and cellular proteins inside the HIV-1 core, yielding a model for the topology of various proteins inside the virion.

L27 ANSWER 28 OF 98 MEDLINE
2001092665 Document Number: 20578211. PubMed ID: 11134289. Organization of immature human immunodeficiency virus type 1. Wilk T; Gross I; Gowen B E; Rutten T; de Haas F; Welker R; Krausslich H G; Boulanger P; Fuller S D. (The Structural Biology Programme, European Molecular Biology Laboratory, D69012 Heidelberg, Federal Republic of Germany.) JOURNAL OF VIROLOGY, (2001 Jan) 75 (2) 759-71. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Immature retrovirus particles contain radially arranged Gag polyproteins in which the N termini lie at the membrane and the C termini extend toward the particle's center. We related image features to the polyprotein domain structure by combining mutagenesis with cryoelectron microscopy and image analysis. The matrix (MA) domain appears as a thin layer tightly associated with the inner face of the viral membrane, separated from the capsid (CA) layer by a low-density region corresponding to its C terminus. Deletion of the

entire p6 domain has no effect on the width or spacing of the density layers, suggesting that p6 is not ordered in immature human immunodeficiency virus type 1 (HIV-1). In vitro assembly of a recombinant Gag polyprotein containing only capsid (CA) and nucleocapsid (NC) domains results in the formation of nonenveloped spherical particles which display two layers with density matching that of the CA-NC portion of immature HIV-1 Gag particles. Authentic, immature HIV -1 displays additional surface features and an increased density between the lipid bilayers which reflect the presence of gp41. The other internal features match those of virus-like particles.

L27 ANSWER 23 OF 98 MEDLINE
2001361665 Document Number: 21315150. PubMed ID: 11423440. HIV -1 capsid protein forms spherical (immature-like) and tubular (mature-like) particles in vitro: structure switching by pH-induced conformational changes. Ehrlich L S; Liu T; Scarlata S; Chu B; Carter C A. (Department of Molecular Genetics & Microbiology, State University of New York at Stony Brook, New York 11794, USA.) BIOPHYSICAL JOURNAL, (2001 Jul) 81 (1) 586-94. Journal code: 0370626. ISSN: 0006-3495. Pub. country: United States. Language: English.

AB The viral genome and replicative enzymes of the human immunodeficiency virus are encased in a shell consisting of assembled mature capsid protein (CA). The core shell is a stable, effective protective barrier, but is also poised for dissolution on cue to allow transmission of the viral genome into its new host. In this study, static light scattering (SLS) and dynamic light scattering (DLS) were used to examine the entire range of the CA protein response to an environmental cue (pH). The CA protein assembled tubular structures as previously reported but also was capable of assembling spheres, depending on the pH of the protein solution. The switch from formation of one to the other occurred within a very narrow physiological pH range (i.e., pH 7.0 to pH 6.8). Below this range, only dimers were detected. Above this range, the previously described tubular structures were detected. The ability of the CA protein to form a spherical structure that is detectable by DLS but not by electron microscopy indicates that some assemblages are inherently sensitive to perturbation. The dimers in equilibrium with these assemblages exhibited distinct conformations: Dimers in equilibrium with the spherical form exhibited a compact conformation. Dimers in equilibrium with the rod-like form had an extended conformation. Thus, the CA protein possesses the inherent ability to form metastable structures, the morphology of which is regulated by an environmentally-sensitive molecular switch. Such metastable structures may exist as transient intermediates during the assembly and/or disassembly of the virus core.

L28 ANSWER 5 OF 5 MEDLINE
89308620 Document Number: 89308620. PubMed ID: 2663848. Purification and characterization of human immunodeficiency virus (HIV) core precursor (p55) expressed in Saccharomyces cerevisiae. Vlasuk G P; Waxman L; Davis L J; Dixon R A; Schultz L D; Hofmann K J; Tung J S; Schulman C A; Ellis R W; Bencen G H; +. (Department of Biological Chemistry, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Jul 15) 264 (20) 12106-12. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The core structure of retroviruses, including the human immunodeficiency virus (HIV), consists of

proteins that are initially synthesized as polyprotein precursors and then processed by a virally encoded protease yielding the mature core polypeptides. **To obtain sufficient quantities of the purified HIV core precursor p55 for detailed studies, a segment of HIV DNA encoding the full length core precursor polyprotein p55 was expressed in Saccharomyces cerevisiae using a plasmid containing a constitutive galactose promoter.** The expression of this DNA produced a protein with an estimated molecular size of 55,000, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE); this protein was immunoreactive to anti-HIV p24 antisera. Following cell lysis, freezing, and thawing, the expressed protein was an insoluble aggregate that served as the starting material for the purification process. **Solubilization of the insoluble p55 with guanidine HCl followed by phenyl-Sepharose column chromatography and high performance liquid chromatography resulted in a preparation of p55 that was greater than 95% pure by SDS-PAGE, immunoreactive to anti-HIV core protein antibodies, and completely soluble in aqueous solution.** The expressed p55 appeared to be myristoylated as evidenced by the incorporation of radiolabel following incubation of recombinant yeast cells with [3H]myristic acid; in addition the amino terminus of the final purified protein was blocked. Proteolytic digestion of purified p55 with synthetic HIV protease yielded the predicted amino- and carboxyl-terminal products; these were confirmed by amino acid sequence analysis. In contrast, digestion of purified p55 by the protease derived from the avian myeloblastosis virus resulted in fragments that were different in size from those produced by the HIV protease. The availability of the purified, full length water-soluble HIV core precursor will be useful in identifying agents that inhibit its processing by the HIV protease.

L29 ANSWER 1 OF 1 WPIDS (C) 2003 THOMSON DERWENT
AN 2001-570777 [64] WPIDS
DNC C2001-169710
TI Screening viral assembly and maturation modulator by triggering assembly of viral structural protein in soluble form, in presence of candidate and control compounds and monitoring increase/decrease in viral assembly.
DC B04 D16
IN PREVELIGE, P E
PA (PREV-I) PREVELIGE P E; (UABR-N) UAB RES FOUND
CYC 26
PI WO 2001066805 A1 20010913 (200164)* EN 48p
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
W: AU CA JP MX NZ
US 2001036627 A1 20011101 (200168)
AU 2001050029 A 20010917 (200204)
EP 1261746 A1 20021204 (200280) EN
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR
ADT WO 2001066805 A1 WO 2001-US40247 20010306; US 2001036627 A1 Provisional US 2000-186981P 20000306, US 2001-800240 20010306; AU 2001050029 A AU 2001-50029 20010306; EP 1261746 A1 EP 2001-923324 20010306, WO 2001-US40247 20010306
FDT AU 2001050029 A Based on WO 200166805; EP 1261746 A1 Based on WO 200166805
PRAI US 2000-186981P 20000306; US 2001-800240 20010306

AB WO 200166805 A UPAB: 20011105
NOVELTY - Screening for a compound that modulates viral assembly (VA) and maturation, involves maintaining viral structural protein (I) in soluble form, triggering assembly of (I), contacting (I) with a candidate or control compound (C1,C2) that inhibit VA, and monitoring VA. An increase or decrease of VA in presence of (C1) compared to (C2) indicates that (C1) promotes or inhibits VA, respectively.
USE - The method is useful for screening a compound that modulates the HIV-1 assembly and maturation (claimed).
ADVANTAGE - The method is rapid in identifying anti-viral drugs.
DESCRIPTION OF DRAWING(S) - The figure shows the HIV capsid assembly.
Matrix protein MA
Capsid protein CA
Nucleocapsid protein NC.
Dwg.1/9